N-FIN-1990

# AD-A230 971

## **Towards Wound Closure Optimization: Final Report**

Larry I. Sanders



LaserSurge, Inc.

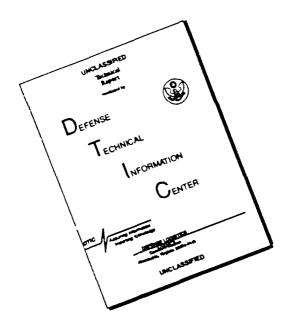
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## **Summary**

Extensive experimentation with many combinations of lasers and chromophores was conducted to determine the optimal parameters for laser tissue welding. New Zealand White rabbit small bowel was used as an in vitro model for this study. The analysis indicates that particular chromophores can be utilized to enhance the effectiveness of certain lasers. For example, India ink is an effective chromophore for the 1.06 micron Nd: YAG laser, while blood is most effective for the 0.514 micron Ar<sup>++</sup> laser. The 2.1 micron Ho: YAG laser produced strong chromogore-ore-free welds, and also produced strong welds in the presence of India ink and blood. The laser welding literature indicates that Indocyanine Green is an adjuvant to laser welding with the 0.808 micron Ga: Al: As diode laser. The diode lasers that were examined in this study did not provide sufficient energy to weld tissue and evaluate these statements. Indocyanine Green did prove to be an effective chromophore for the 0.532 micron KTP laser. The apparatus designed and developed for this study provides an unique method to determine the laser tissue welding parameters which will produce the strongest welds.

#### Introduction

LaserSurge, Inc, of Rochester, NY was established to develop, demonstrate, and facilitate the clinical use of automated surgical laser delivery systems. The speed, effectiveness, and reliability of using laser energy to connect or weld living tissue makes laser tissue welding one of the most promising areas of medical research today. The controlled application of laser energy thermally induces intrinsic tissue changes which lead to both immediately strong bonds between tissue and rapid restoration of tissue function. Conventional surgical techniques typically require sutures and staples to close tissue wounds or to construct anastomoses (i.e., surgical connections that provide functional communication between hollow organs such as bowel). A surgeon's bonding of tissue using laser tissue welding can be compared to a metal worker's handling of steel. Whereas the conventional suturing or stapling is like the use of screws or rivets in steel, laser tissue welding, like welding steel, uses an outside energy source to cause intrinsic material changes which alone provide for the strong bond. A fundamental requirement to weld either material is that the surfaces to be welded be in intimate contact with each other. To fully realize the enormous potential of this revolutionary wound closure modality, an enhanced theoretical understanding of laser tissue welding is essential. In an effort to better recognize and evaluate the many factors that determine a successful laser tissue weld, LaserSurge has developed a laser tissue welding equation which describes laser tissue welding as a function of its component parameters; lasing parameters, chromophores, and tissue parameters. Using this structured approach, an investigator can readily dissect and compare the effects of changes in individual or multiple variables. This study was designed to identify the most favorable laser tissue welding conditions. To accomplish this goal, an integrated laser tissue welding and tensiometry (weld strength measurement) system was designed, constructed, and tested. This system permits the user to selectively vary several parameters of interest including welding temperature, welding time, aperture size, laser type, power, and chromophores. Experimental trials using different combinations of the aforementioned parameters were performed in this study.





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## Methods, Assumptions, and Procedures

#### Methods

#### **Device Refinement and Evaluation**

In order to examine the effects of multiple combinations of lasers and chromophores, a new device was developed to quantify the strength of laser welds. The laser welding/tensiometry apparatus permits any laser which can be transmitted through an optical fiber to be coupled to the beam alignment system. The beam alignment system configured the beam so that it slightly overfilled the aperture. The beam passed through a high-speed shutte. Sich opened and closed in response to the thermocouple temperature reading. The aperture size was fixed at either  $0.5 \times 4.0 \, \text{mm}$  or  $2.0 \times 4.0 \, \text{mm}$ , and the aperture was placed just above the surface of the tissue strips to be welded. The beam impinged perpendicularly upon the tissue strips at the site of apposition. A thermocouple was placed between the strips so that it was positioned 1 mm beneath the surface of the weld. The strips were then brought into contact by a piezoelectric motor under computer control. The laser welding temperature control program allowed the operator to designate the parameters of interest, including the welding control temperature. Following welding, the welded tissue was pulled apart and the load required to rupture the weld was measured as a function of the distance moved. Graphs of lead versus distance were generated to determine maximum weld strength.

The tensiometry software was seamlessly integrated into the laser welding control program by Mr. Williar Kraft. This new program eliminated the need to reboot the computer between experimental trials. This major improvement permitted more rapid data acquisition and reduced the average time between trials to approximately five minutes.

The thermocouple placement system has also been modified to provide more reproducible and accurate temperature measurements. A single thermocouple was mounted on a specially-designed plexiglas holder. This thermocouple was placed 1 mm below the surface of the apposed tissue at the site of welding, as depicted in Figure 1 in Appendix C. The temperature recorded from the thermocouple controlled the opening and closing of the shutter mechanism, thereby maintaining the designated tissue welding temperature. The new thermocouple holder permits easier, faster, and more reliable thermocouple placement.

A new problem surfaced during the experimentation. The stages began to stall and resist free movement because of dried bowel and saline which had entered the stage. The stages were removed, cleaned, and remounted. Periodic cleaning and maintenance have rectified this problem.

## **Assumptions**

An implicit assumption in the original design of this experimental protocol is that the freshly harvested in vitro bowel very closely resembles native in vivo bowel. The time from resection to welding was minimized by sequentially harvesting small sections of bowel from intravenously anesthetized rabbits. The resected bowel was placed in physiological saline to maintain its integrity, while the remaining intact bowel was returned to the

abdomen for later use. Tissue processing and handling were minimized to the extent possible. Tissue trauma was minimized by handling and manipulating the bowel gently. We have assumed that the in vitro tissue would not be significantly altered by the removal and processing steps. The veracity of this assumption is important since the intent of this study is to generate data which will be readily transferrable to clinical applications.

One set of experiments suggests that the in vivo bowel model may not be wholly representative of the in vivo state. When more than one hour elapsed between bowel resection and welding, the welds produced were clearly inferior to welds produced using freshly harvested bowel under the same conditions. This lack of success may be attributable to changes in tissue turgidity and perhaps also to other biological effects (platelet aggregation and adhesion, etc.). The bowel turgidity appears to change as a result of storage in physiological saline. It is unclear whether this change might influence the laser welding of bowel. Ischemia and oxygen deprivation may also cause changes which are responsible for the difference seen between welding in vitro and in vivo.

As a consequence of the <u>in vitro</u> alterations to bowel, the weld strengths obtained in this study were much lower than anticipated based upon our earlier <u>in vivo</u> data. In order to obtain recognizable welds, the highest laser power and temperature settings were employed. Several of the lasers, namely the diode and Argon<sup>\*+</sup>, were unable to provide the high power output required to weld tissue. Thus, the results for the diode and Argon lasers are inconclusive, and few or no welds were performed using these lasers.

## **Experimental Procedure**

The experimental protocol required the resection of small bowel from New Zealand White rabbits. As discussed in the third quarter 1990 report, weld strength appears to diminish as the length of time between bowel harvesting and welding increases. In order to more closely emulate the clinical setting, fresh bowel was sequentially resected from an intravenously anesthetized rabbit (see Figure 2 in Appendix C for a complete description of the experimental protocol). The harvested tissue was cut into strips measuring 4 mm x 20 mm. The strips were carefully positioned on the laser welding/tensiometry apparatus. The thermocouple was positioned 1 mm deep at the site to be welded and the tissue strips were brought into apposition. Next, the laser was fired and, following the laser exposure, a welded tissue seam was created where the tissue strips were in contact. Thus, the thermocouple was imbedded in the welded tissue. The laser welding temperature control software directed the opening and closing of the shutter to maintain the designated welding temperature. Immediately following laser welding, the tissue was pulled apart and the load required to break the bond (in grams) was measured as a function of the distance (in millimeters) moved. Load versus distance plots were generated to assess the strength of each weld. Maximum strength was determined for each weld.

The 0.808 micron diode lasers used in this study were not powerful enough to use for laser welding using the apparatus as designed. Both LaserSurge's Spectra Diode Laboratory 3 W laser diode array and Columbia-Presbyterian's 1.5 W IRIS Medical OcuLight SL diode were coupled to the laser welding/tensiometry apparatus. The power delivered to the weld site, as measured by a power meter, was insufficient to weld tissue, since less than 0.15 W was transmitted through the optical delivery system. No appreciable temperature rise was observed, even with the Indocyanine Green chromophore. Thus, further studies of the diode laser and its interaction with chromophores were not performed.

Another laser which was initially included in this study was the 1.32 micron Nd:YAG. Rochester General Hospital has one 1.32 micron laser, and it had not been used in more than one year. When the laser was turned on, the laser's power setting mechanism did not function. This laser was unable to be repaired prior to the conclusion of this study. Thus, the 1.32 micron Nd:YAG laser was not studied further.

The Rochester General Hospital 0.514 micron Ar<sup>++</sup> laser was studied. This laser, a 7.5 W Lexel Aurora Model 150 Pump Laser and a Model 600 Dye Laser, is a tremendously large research unit. The delivered power was quite low, approximately 0.7 W, and this precluded testing of some chromophore and temperature combinations.

The 100 W, CW, Sharplan 2100 1.06 micron Nd:YAG laser was examined using all chromophores and both aperture sizes. The maximum power of 5.0 W (delivered to the site of welding) was selected for use throughout since, at the lower temperatures even with this high power, weld strength was low. Thus, the lower power settings were not examined.

The Columbia-Presbyterian Medical Center in New York, NY has a Coherent Two Point One Holmium laser, with a maximum output power of 15 W operating in pulsed mode. The 2.1 micron Ho:YAG was examined at approximately 1 and 5 W with the small and large apertures.

The 20 W, CW, LaserScope 0.532 KTP laser was also examined in this study. Both aperture sizes were examined at the highest obtainable delivered power of 4 W.

The power delivered to the weld site through the optical system was occasionally not sufficient to attain some of the higher temperatures. These results are indicated in the data section that follows.

#### Results and Discussion

#### Results

The data collected and included in the subsequent analysis are limited in number. Most of the early data collected using the ASYSTANT data acquisition program has been excluded due to problems with the tensiometry apparatus, as will be explained in the Discussion (see Appendix B for these force versus distance plots). A great deal of time was devoted to software development and testing, and therefore, the results reported herein generally come from one or two samples. A thorough statistical analysis was not performed; rather, averages were obtained for each laser and chromophore combination. These averages were assessed to determine the most promising combinations. The averages are presented in Table 1.

## TABLE 1- MAXIMUM WELD STRENGTH (g)

_	(Small Aperture, 0.7-	•			
<u>TEMP</u>	BLOOD	<u>ICG</u>	<u>INDIA INK</u>		
UNWELDED	0.9 1.2	0.6 0.7	0.2		
50° C			1.1		
60° C	1.4	1.0	0.9		
70° C	-	0.7	1.3		
80o C	3.8	•	-		
100°C	-	-	-		
0.532 Micron KTP (	Small Aperture, 4.0 W	delivered)			
TEMP	BLOOD	<u>ICG</u>	<u>INDIA INK</u>		
UNWELDED	0.7	0.1	0.5		
50° C	1.7	1.0	0.4		
60° C	1.1	2.2	0.5		
70° C	1.7	2.7	0.9		
80° C	1.3	1.3	1.0		
100° C	2.1	3.4	0.6		
0.532 Micron KTP (Large Aperture, 4.0 W delivered)					
<u>TEMP</u>	<b>BLOOD</b>	<u>ICG</u>	<u>INDIA INK</u>		
UNWELDED	-	0.1	-		
50° C	-	0.7	~		
60° C	-	0.8	-		
70° C	-	1.0	-		
80° C	-	1.0	-		
100° C	-	3.7	-		
1.06 Micron Nd: YAG (Small Aperture, 5.0 W delivered)					
<b>TEMP</b>	BLOOD	<u>ICG</u>	<u>INDIA INK</u>		
UNWELDED	0.2	0.1	0.5		
50° C	0.9	0.9	1.0		
60° C	1.5	1.1	0.7		
70° C	1.6	1.2	2.8		
80° C	1.0	1.0	2.0		
00 C	1.9	1.0	2.0		

1.06 Micron Nd:YAC	G (Large Aperture, 5.0	W delivered)		
<b>TEMP</b>	BLOOD	<u>ICG</u>	<u>INDIA INK</u>	
UNWELDED	-	-	1.0	
50° C	-	-	1.0	
60° C	-	-	1.0	
70° C	-	-	1.0	
800 C	-	-	1.25	
100° C	-	-	2.0	
2.1 Micron Ho:YAG	(Small Aperture, 5.0	W delivered)		
<b>TEMP</b>	BLOOD	<u>ICG</u>	<u>INDIA INK</u>	NO CHROMO
UNWELDED	0.6	1.7	0.6	0.7
500	1.8	3.4	5.6	4.0
60°	3.7	2.9	9.4	5.8
70°	4.8	9.6	7.0	7.9
80o	13.6	5.9	21.0	8.9
100°	29.4	10.9	21.0	20.8
2.1 Micron Ho:YAG	(Large Aperture, 5.0	W delivered)		
<b>TEMP</b>	BLOOD	<u>ICG</u>	<u>INDIA INK</u>	NO CHROMO
UNWELDED	0.6	0.9	1.0	0.8
50° C	0.6	4.0	5.4	13.9
60° C	1.5	11.4	2.4	1.1
70° C	2.8	2.8	15.3	2.8
80o C	31.3	45.0	14.2	24.0
		43.0	14.2	34.0
100° C	36.5	50.8	2.1	39.0
	36.5 (Small Aperture, 1.0	50.8		
	(Small Aperture, 1.0 BLOOD	50.8 W delivered) <u>ICG</u>	2.1 <u>INDIA INK</u>	39.0 <b>NO CHROMO</b>
2.1 Micron Ho:YAG	(Small Aperture, 1.0 BLOOD 1.3	50.8 W delivered) ICG 0.8	2.1 <u>INDIA INK</u> 1.6	39.0 NO CHROMO 1.2
2.1 Micron Ho:YAG TEMP	(Small Aperture, 1.0 ) <b>BLOOD</b> 1.3  2.1	50.8 W delivered) ICG 0.8 2.1	2.1 <u>INDIA INK</u> 1.6 1.7	39.0  NO CHROMO 1.2 1.7
2.1 Micron Ho:YAG TEMP UNWELDED	(Small Aperture, 1.0 BLOOD 1.3	50.8 W delivered) ICG 0.8	2.1 <u>INDIA INK</u> 1.6	39.0 NO CHROMO 1.2
2.1 Micron Ho: YAG TEMP UNWELDED 50° C	(Small Aperture, 1.0 ) <b>BLOOD</b> 1.3  2.1	50.8 W delivered) ICG 0.8 2.1	2.1 <u>INDIA INK</u> 1.6 1.7	39.0 NO CHROMO 1.2 1.7

5.9

17.9

2.9

9.5

100° C

2.1 Micron Ho: YAG (Large Aperture, 1.0 W delivered)

<b>TEMP</b>	BLOOD	<u>ICG</u>	INDIA INK	NO CHROMO
UNWELDED	1.0	0.3	0.2	0.2
50° C	6.7	2.7	1.3	1.5
60° C	6.1	6.8	0.9	-
70° C	5.5	24.5	2.0	4.4
80° C	4.9	4.1	0.8	1.4
100° C	11.3	5.3	3.1	4.1

#### NOTES:

BLOOD = fresh whole blood collected in a Vacutainer blood collection tube with heparin. ICG = prepared as a 2X solution (25 mg Indocyanine Green per 10 ml aqueous solvent).

<sup>- =</sup> unable to perform.

#### Discussion

#### Thermocouple Placement

The earlier observation that thermocouple placement is crucial continued to be supported by experimentation. If the thermocouple is placed above the weld site, the recorded temperature is an artefact which corresponds to the heating of the thermocouple and not to the heating of the tissue at the weld site. If the thermocouple is placed too deeply, it again fails to accurately represent the temperature increase at the site of welding since the recorded temperatures are lower than those at the weld. Since the laser welding temperature control program controls the temperature at the weld site based upon the thermocouple measurements, proper thermocoupie placement is imperative. The final thermocouple holder design provides adequate reproducibility. When thermocouple placement was incorrect, as evidenced by either an abnormally short or a protracted time to attain the stipulated control temperature, the trial was repeated with fresh tissue strips.

Prior to the design and construction of the new thermocouple holder, there were major problems with thermocouple placement. The thermocouple was difficult to position reproducibly, and thus, much of the early data is erroneous. Since the temperature control program utilizes feedback from the thermocouple, and since the thermocouple was incorrectly positioned, the time of laser exposure as well as the actual temperature at the weld site were incorrect as well.

### **Chromophore Delivery and Placement**

Precise chromophore placement was another important factor in this study. Each of the chromophores tested was a liquid. Attempts were made to deposit the chromophore at the weld site using a micropipettor. This allowed a fixed amount of chromophore to be deposited, but the application was not evenly distributed along the weld seam. A cotton-tipped applicator was used to apply a barely visible coat of chromophore to both tissue strips prior to bringing the strips into apposition. The strips were then brought into contact by the computer-controlled piezoelectric motor. When the requisite apposition was obtained, the top surface of the weld site was gently wiped with a clean cotton-tipped applicator to remove any chromophore which oozed upwards when the tissue was compressed. When too much chromophore was placed at the top of the weld site, excessive energy was absorbed at the surface, rather than in the deeper areas of the weld site, and smoking, charring, and diminished weld strength were noted. Chromophore placement in the deeper half of the weld site resulted in stronger, more homogeneous welds.

## Maintenance of equipment, software package, and its effect on data.

Maintenance of the laser welding/tensiometry apparatus was crucial. The stages upon which the tissue is clamped are highly susceptible to stalling if they become contaminated with dried bowel and saline. Stalling of the motor due to a contaminated stage was readily apparent on the first control run of the day, as the tensiometry plots of force versus distance indicated the resistance of the stage to movement. Periodic removal of the stages for cleaning and maintenance was a necessity. The outside surfaces of the laser welding/tensiometry apparatus were carefully cleaned after each use. These steps prevented corrosion of the apparatus.

Initially, however, the problem of stalling was not so readily apparent. Using the original ASYSTANT data acquisition software package, we were unable to obtain plots of the data in either real time or immediately after the completion of the trial. To plot data in ASYSTANT required a lengthy process of file conversion and computer rebooting. Thus, data was not analyzed until after the animal had been sacrificed. This software problem resulted in the generation of tremendous amounts of erroneous data (see Appendix B), since it was impossible to interpret the effect of stage resistance on the measured weld strengths.

Since there were substantial problems with the software, several different software programs were utilized during this study. ASYSTANT was utilized for the earliest data acquisition, but it was cumbersome and inefficient. The time between welding and tensiometry was often more than five minutes. The final version of the temperature control and tensiometry program is written in BASIC and can switch from the welding temperature control program to tensiometry in a matter of seconds. The data acquired using the BASIC programs was imported into either Lotus 1-2-3 version 2.1 or Lotus Symphony release 2.0 for subsequent analysis and graphing.

All data were collected at the laboratory and were stored on 3.5 inch high density floppy disks to permit data analysis at the office. Additional backup copies of the data have been made for the LaserSurge software archives in case of loss or damage to the working disks. These disks are stored off site.

# Overall results- difference in aperture size and its effect, power delivered and its effect, chromophores and their effect, time of welding and its effect

Aperture size was one parameter examined in this study. Two aperture size. were utilized, 0.5 x 4.0 mm and 2.0 x 4.0 mm. The small aperture, with its attendant higher power density, generally produced stronger welds. This was true for all lasers examined, except for the Ho:YAG. For the 5.0 W Ho:YAG trials, the large aperture produced better welds with either Indocyanine Green or in the absence of chromophore. For the 1.5 W trials, the large aperture again produced stronger welds with Indocyanine Green, blood, and without a chromophore.

Power densities can also be calculated for the two aperture sizes used. The power densities, in W/cm<sup>2</sup>, were:

- Small Aperture, 1 W- 50
- Small Aperture, 5 W- 250
- Large Aperture, 1 W- 12.5
- Large Aperture, 5 W- 62.5.

Since the large aperture appeared to weld better than the small aperture for the Ho:YAG laser, perhaps the increased surface area of welding was responsible for the strong welds seen.

High and low power settings were also examined. The higher power of 5 W produced welds of superior strength when compared to the lower power welds. This is true for equal welding temperatures, and seems surprising since the lower power setting often required a longer total laser exposure to reach the desired welding temperature.

The effect of the length of exposure to laser energy on weld strength was not assessed in this study. Most of the temperature settings used required between 15 and 30 seconds to reach the designated control temperature. When the control temperature was attained, feedback from the thermocouple to the shutter control maintained the desired temperature for 10 s. Since there was significant variation in the amount of time required to reach the predetermined welding temperature, the differences due to the variation of controlled exposure would be very difficult to interpret. Thus, a single, fixed 10 s exposure was consistently used throughout the study.

The relationship between maximum temperature and weld strength is also unclear. The highest temperature examined, 100 degrees C, usually elicited smoking as well as tissue blanching and desiccation. Occasional charring was noted in some samples. As mentioned above, the time required to reach the desired temperature varied from sample to sample. Thus, the conclusion that weld strength appears to increase with increasing temperature must be tempered with caution. This is particularly true because the 100 degree welds were often slightly weaker than the 80 degree welds. We speculate that this might be due to increased thermal injury at the higher temperature. Many samples that were welded at 100 degrees became charred or carbonized, and the tissue was dry and brittle. This type of extreme thermal injury may compromise the strength of the weld, and may explain why the 100 degree welds were weaker than the 80 degree welds. Many of the 100 degree welds that were mildly desiccated and blanched seemed to be strongly welded. The overall pattern seemed to indicate that weld strength did increase with increasing temperature.

#### Conclusions

Laser tissue welding is successful when the correct combinations of laser wavelength, chromophore, and aperture size are utilized. Based upon the <u>in vitro</u> results obtained in this study, the following laser/chromophore combinations appear to show promise for laser tissue welding:

- 0.514 micron Ar<sup>++</sup>- blood or ICG chromophore
- 0.532 micron KTP- blood or ICG chromophore
- 1.06 micron Nd:YAG- India ink chromophore
- 2.1 micron Ho: YAG-blood, India ink, or no chromophore.

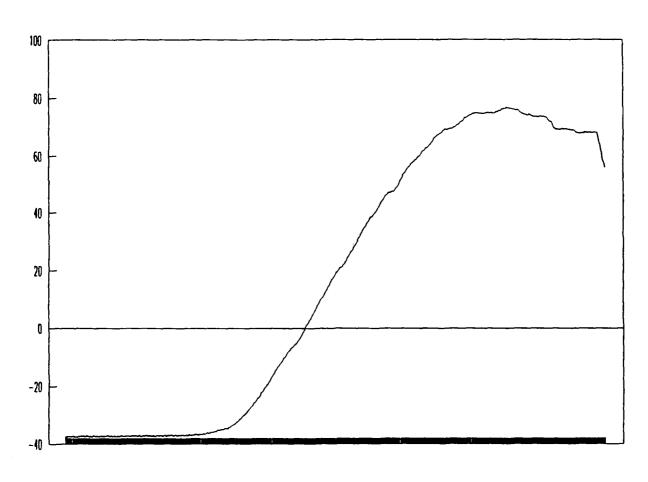
In comparing the weld strength produced by each of these lasers, the Ho:YAG laser clearly produced the strongest welds. Many of the Ho:YAG welds appeared to be full thickness welds. The optimal welding temperature for any of the tested laser and chromophore combinations appeared to be between 80 and 100 degrees C. The higher power levels seemed to produce stronger welds, at least in the range of 1 to 5 W. A certain minimum power is required to increase the tissue temperature to the desired range (80 to 100 degrees C). Whether additional power above this threshold actually improves the strength of the welds cannot be asserted from the data collected.

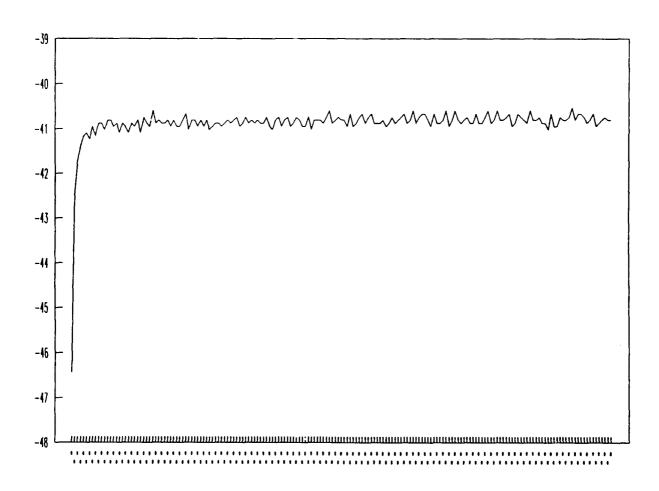
#### Recommendations

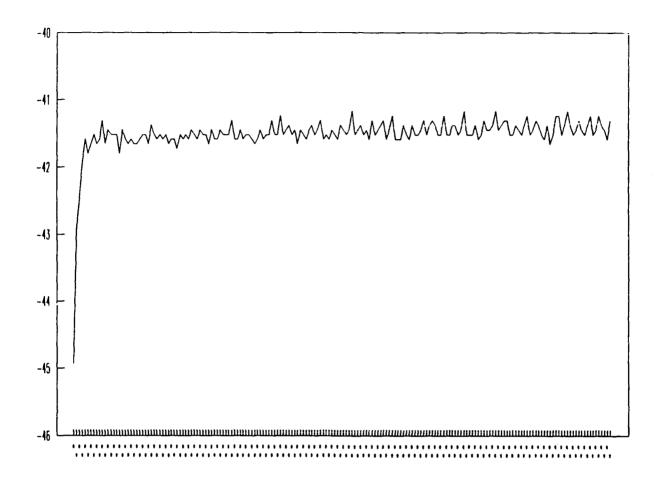
The laser welding/tensiometry apparatus developed for this study provides an unique and ideal method to analyze the effects of various parameters on weld strength. The <u>in vitro</u> model has some limitations, and an <u>in vivo</u> corroboration of these results is needed. An <u>in vivo</u> model would permit an assessment of the effects of thermal injury and healing, two clinically relevant issues.

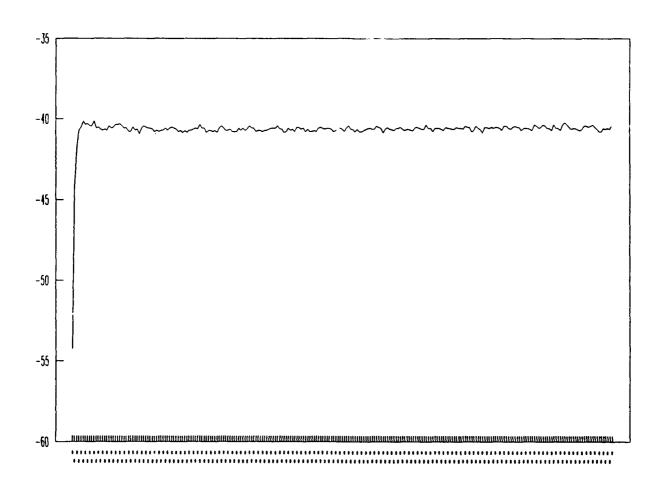
Laser tissue welding continues to show great promise for clinical application, and the data generated in this study provide a solid foundation for future pre-clinical and clinical investigations of this new modality.

# Appendix A- Included Data

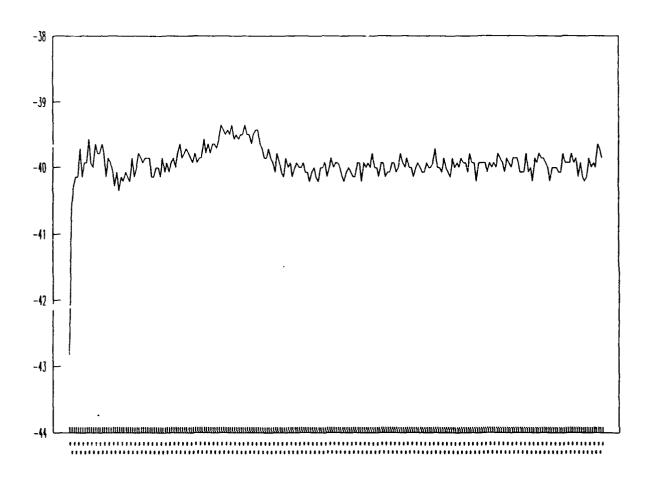




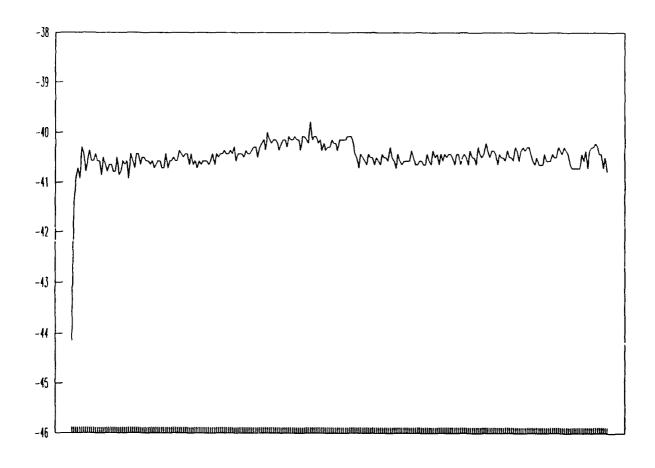




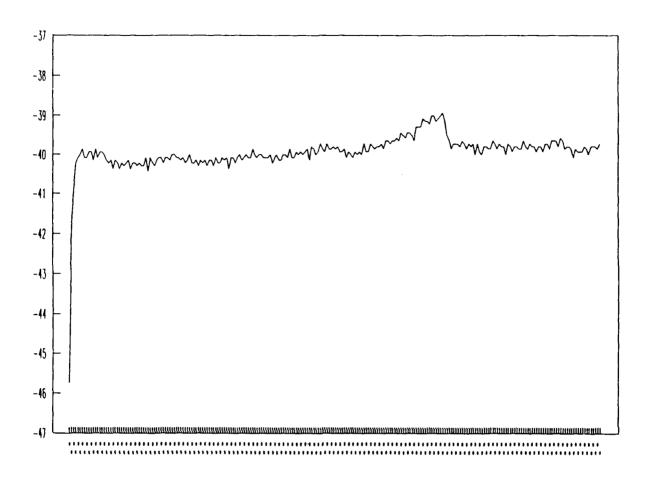
INDIA INK-CONTROLLED AT 70°C



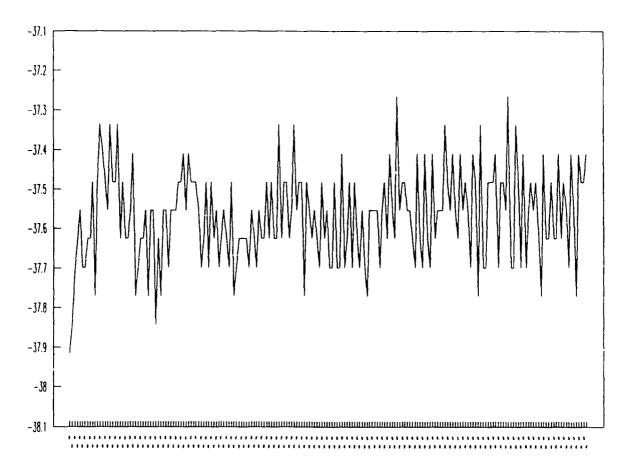
INDIA INK CONTRULLED AT 80°C



INDIA INK CONTROLLED AT 100°C

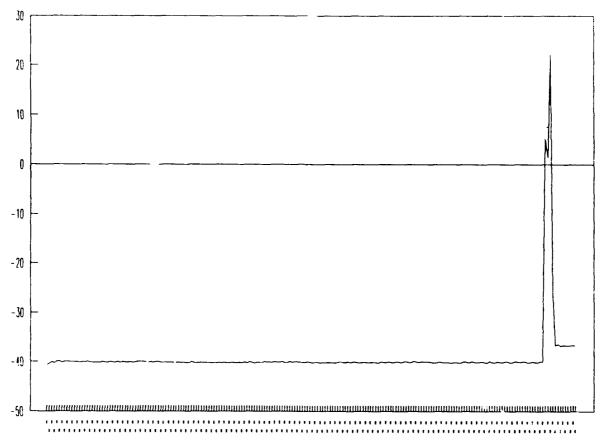


# BLOOD UNWELDED CONTRUL

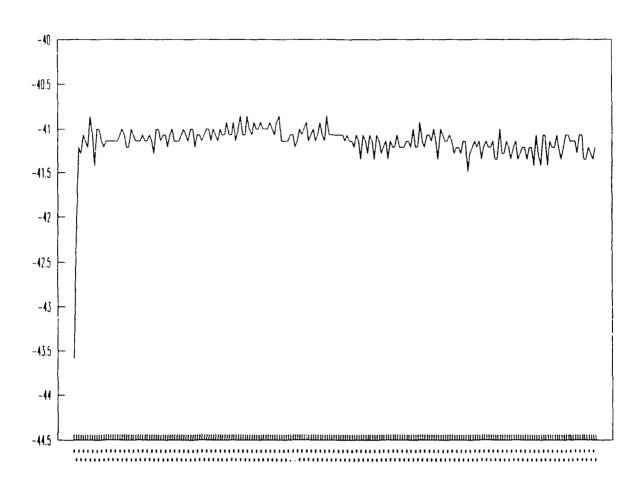


BLOGD CONTRULLED AT 500

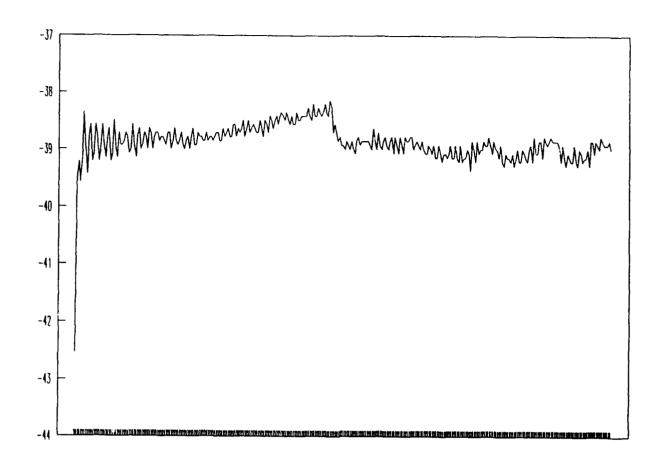
(touched table at end of run)



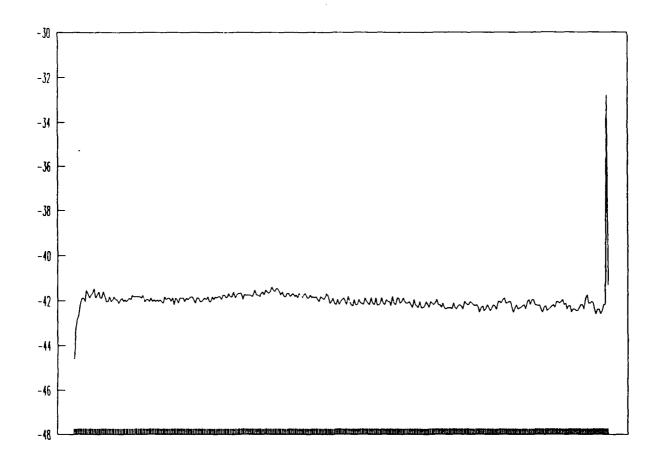
## BLOOD CONTRULLED AT 60°C



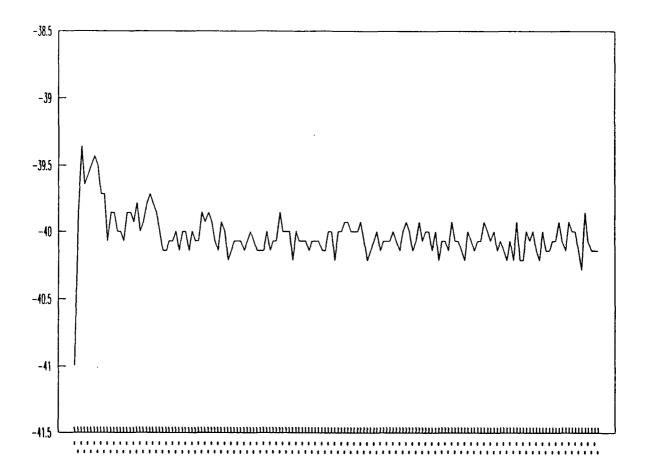
# BLOCK CONTROLLED AT 70°C



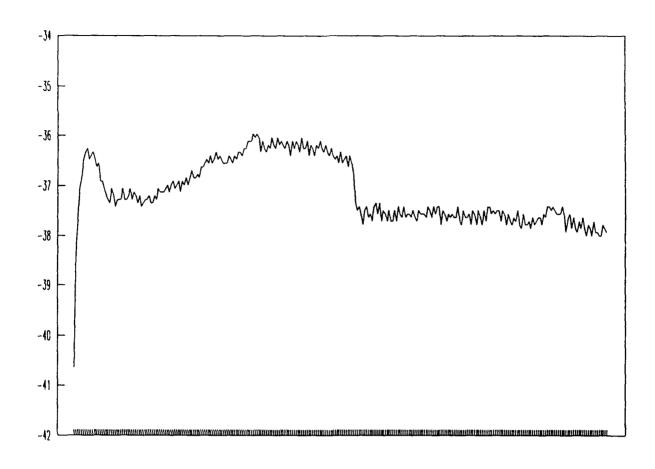
# BLUOD CONTRULCED AT 800



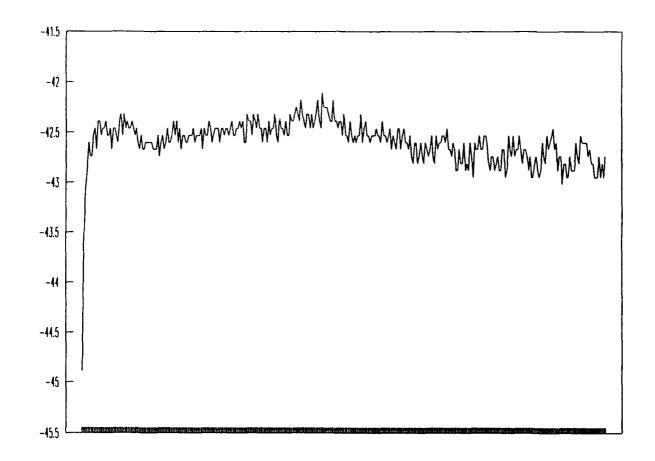
BLOOD CONTRUCCED AT 100°C

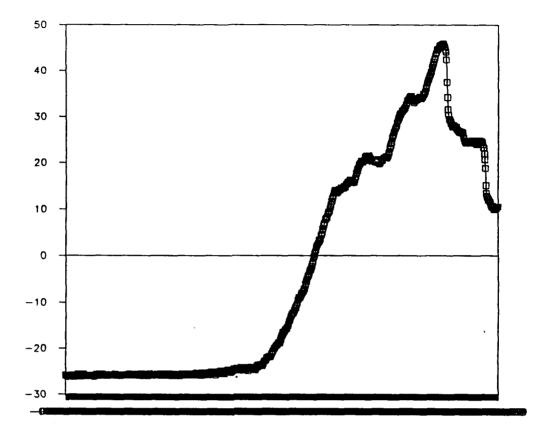


## BLOOD CONTRULLED AT 100°C

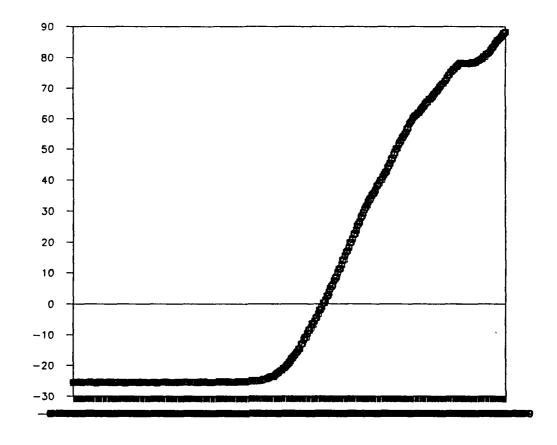


BLOOD CONTRULTED AT 100°C

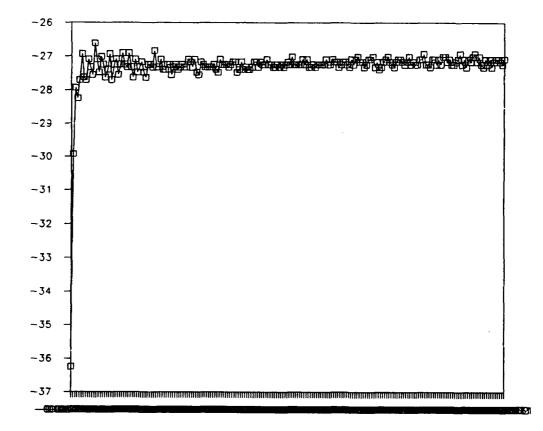


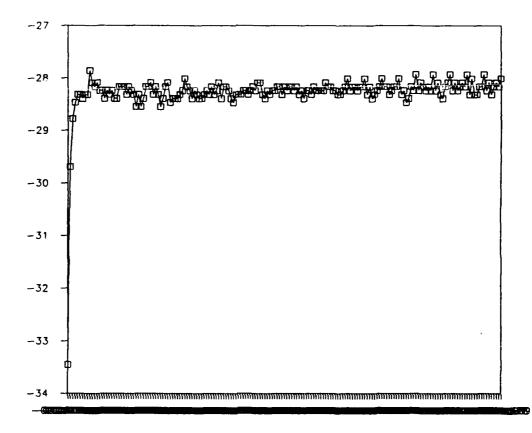


ONE STRIP ICG UNWELDED CONTROL

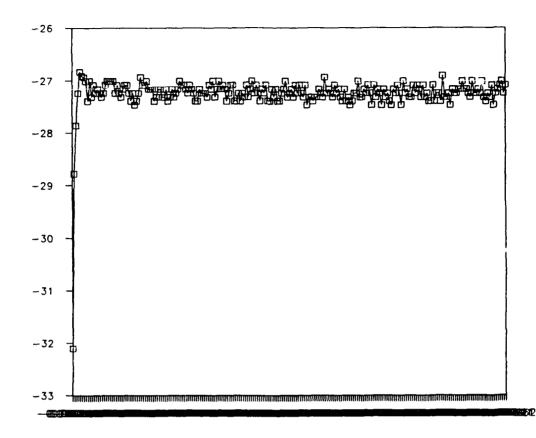


# TWO STRIP UNWELDED CONTROL

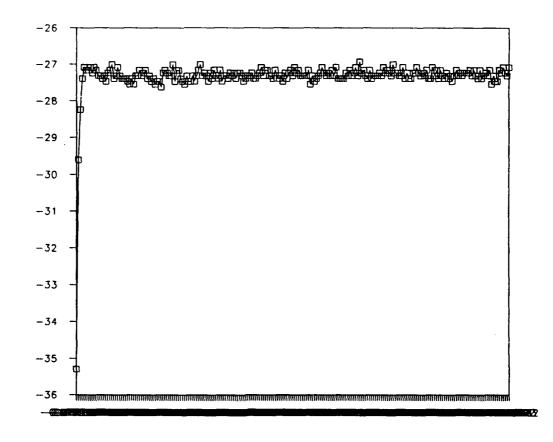




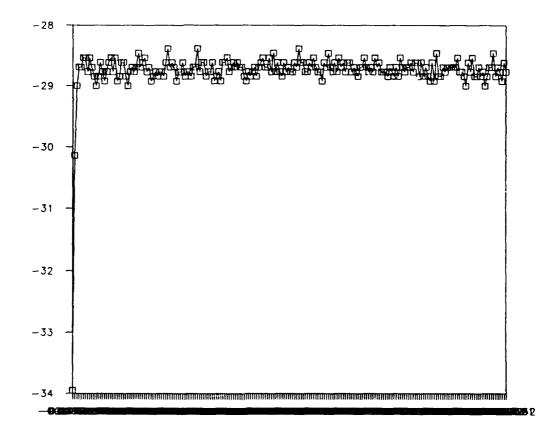
## ICG CONTROLLED AT 50°C



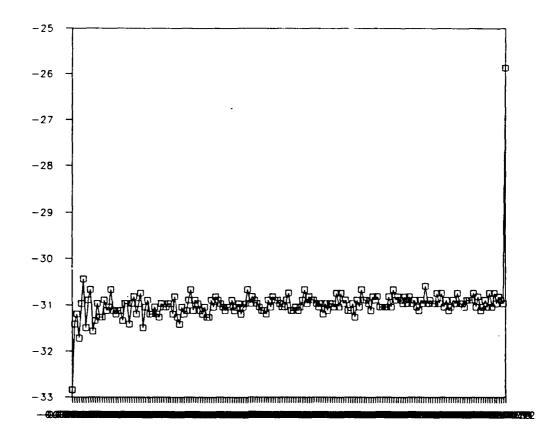
### ICG CONTROLLED AT 5000 6000

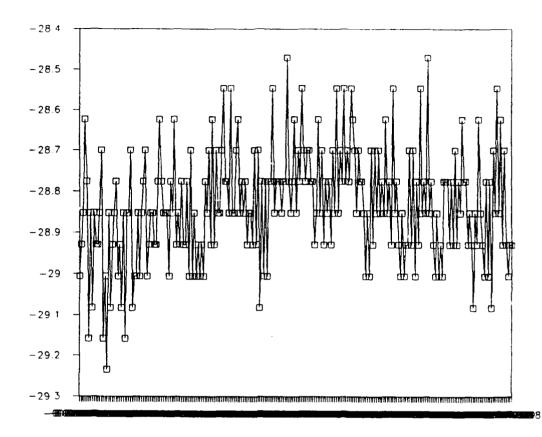


5mall aperture ICG CONTRULLED AT 70°C

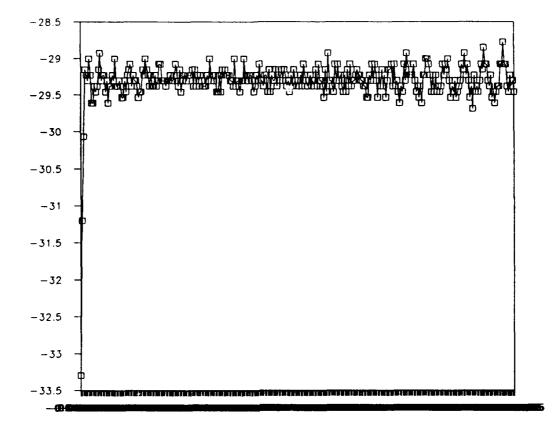


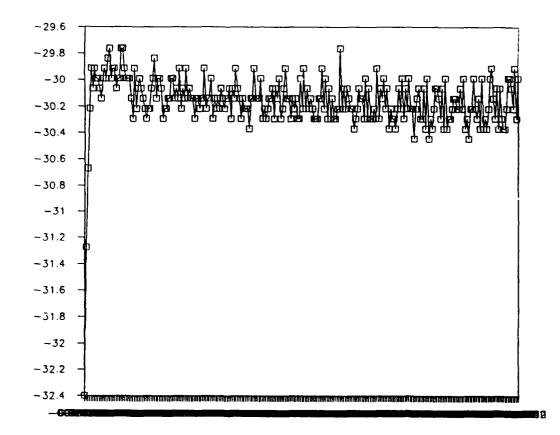
### ICG CONTROLLED AT 80°C



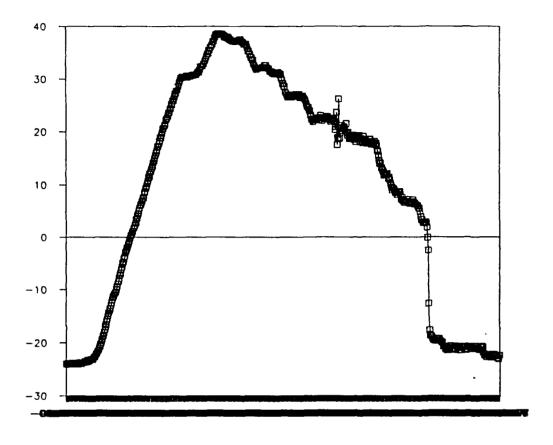


### ICG CONTROLLED AT 100°C





# ONE STRIP CONTROL

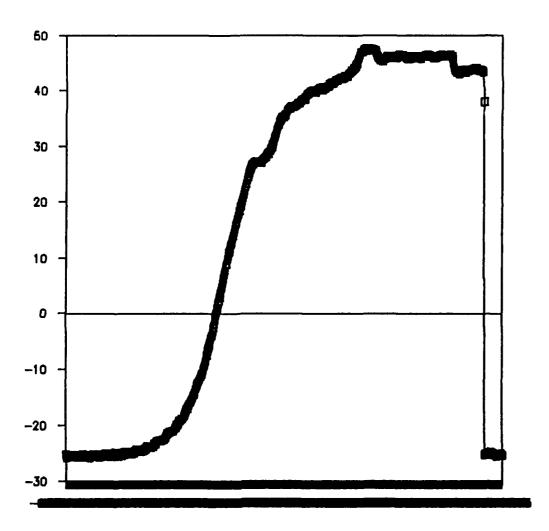


## OCT 9 1990

KTP laser > 20W out of laser > 4.0 W delivered Small Aperture

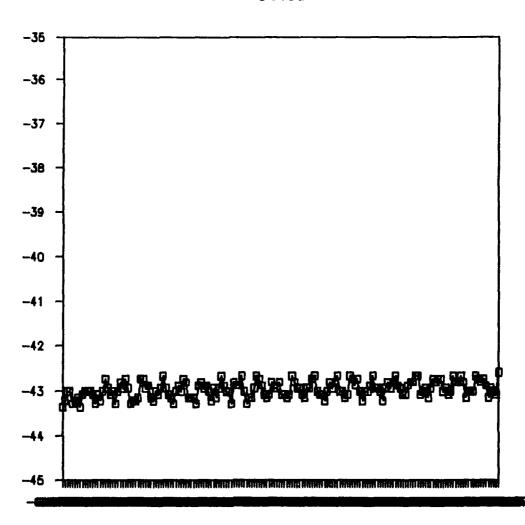
Ferronyl - 19 Ferronyl 3ml 0,9% Saline

Ferrony + Blood - 19 Ferrony | 3 ml whole blood in EDTA



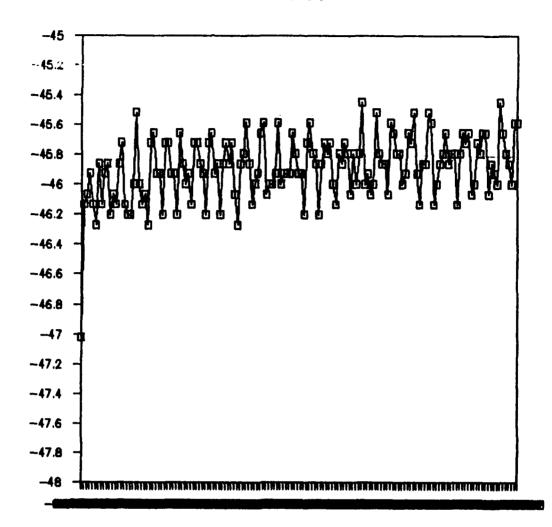
KTP- one stup unnelded

small aperture

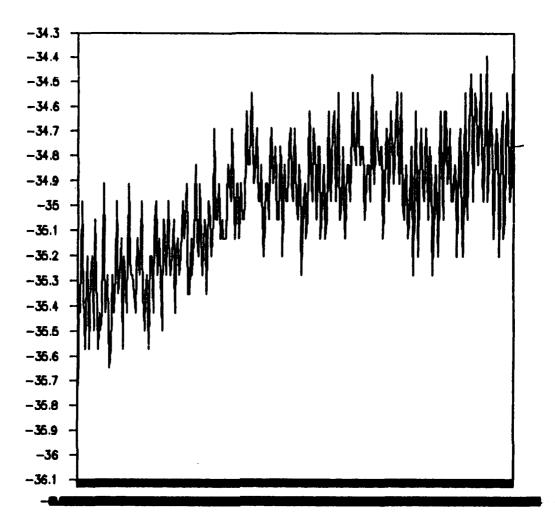


KTP- two strip unwelded control

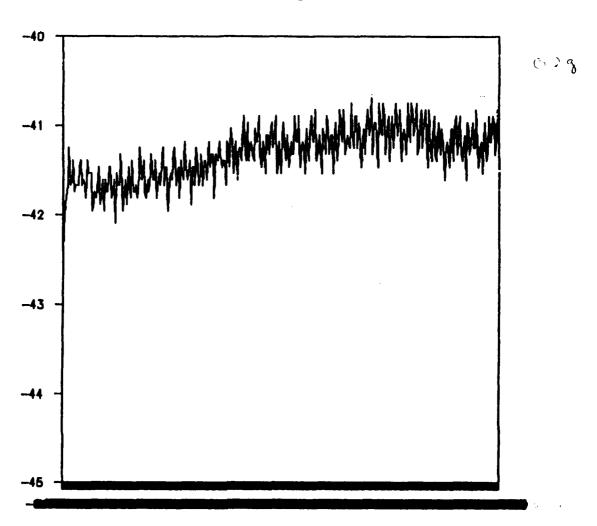
small aperture



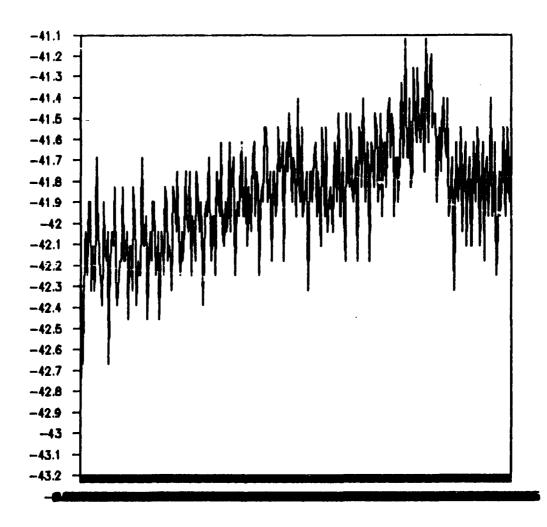
KTP- two slup India ink unwelded control small aperture



KTP- India int-50°C small aperture

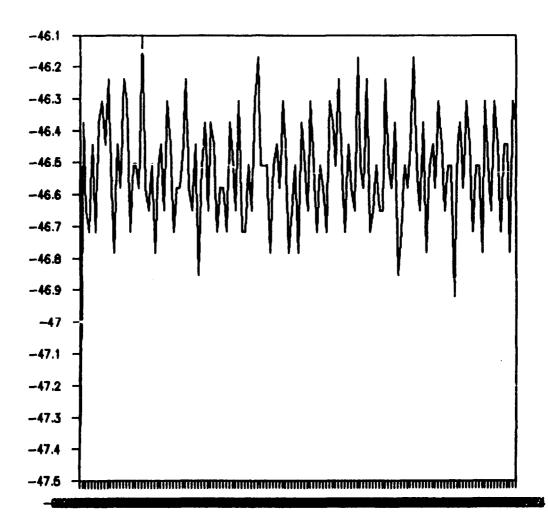


KTP- India int-60°C small aperture



KTP- India ink-70°C small aperture

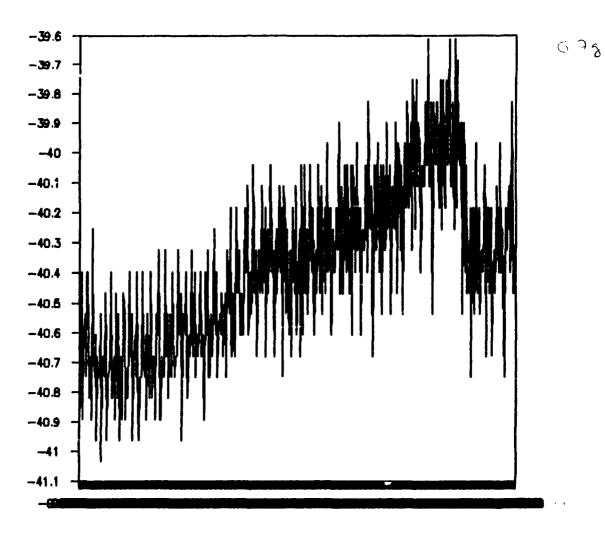
03



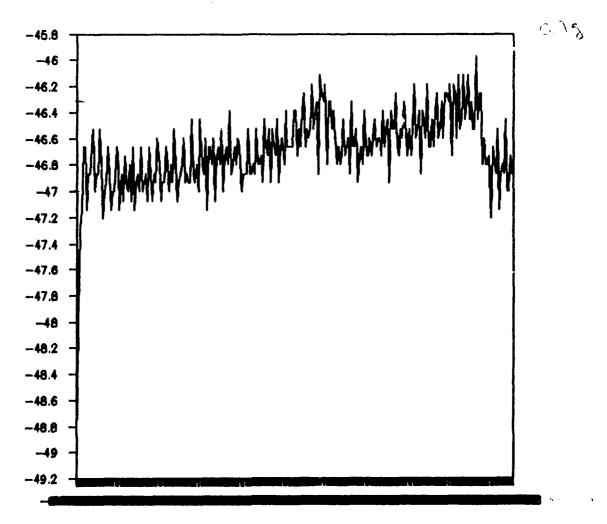
KTP- India ent - 80°C

To too superficial?

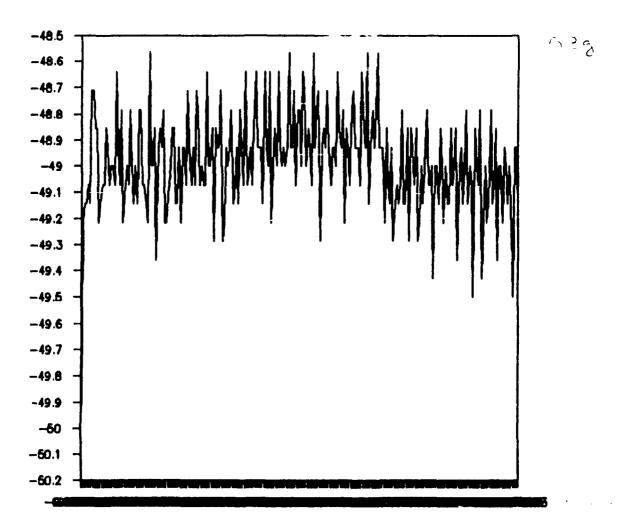
Small aperture



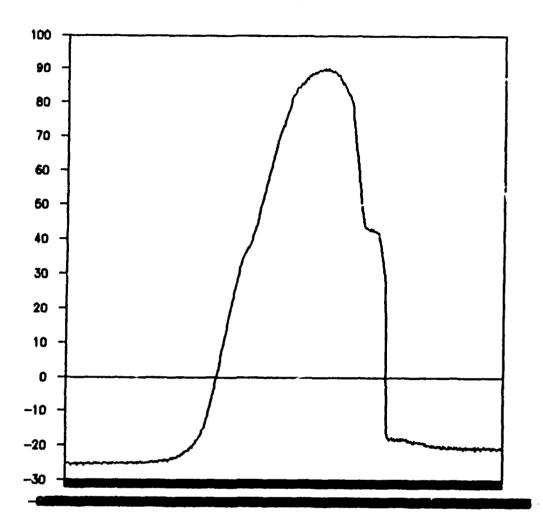
KTP- India inh-80°C small aperture



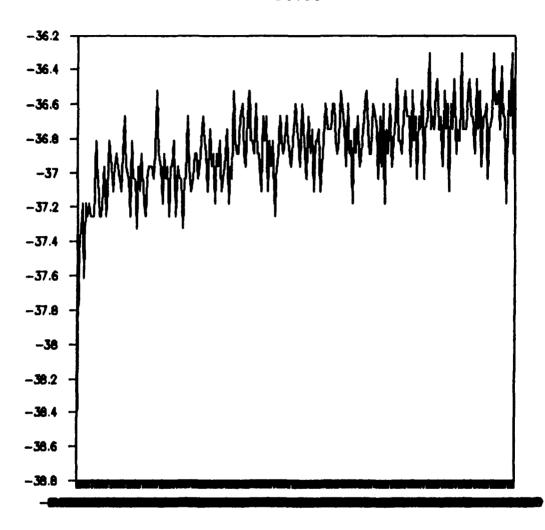
KTP- India int-100°C Small aperture



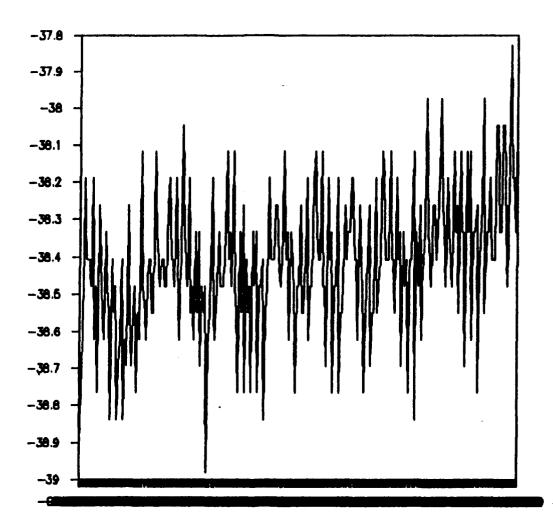
KTP- India un - 100°C small aperture



KIP - one stup control small aperture

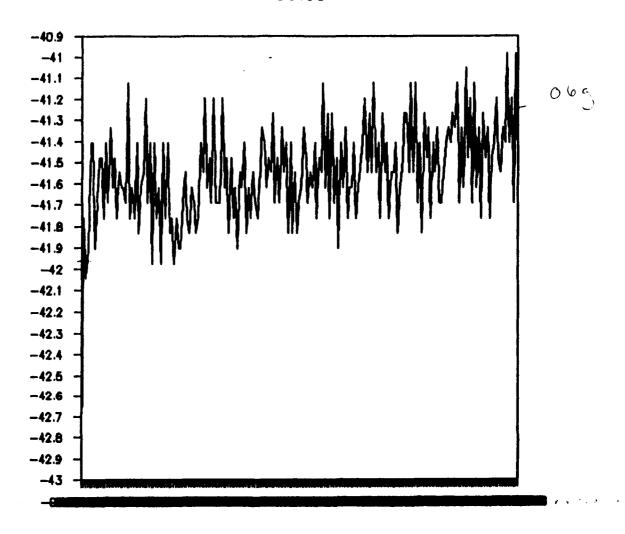


KTP- two strip control small aperture

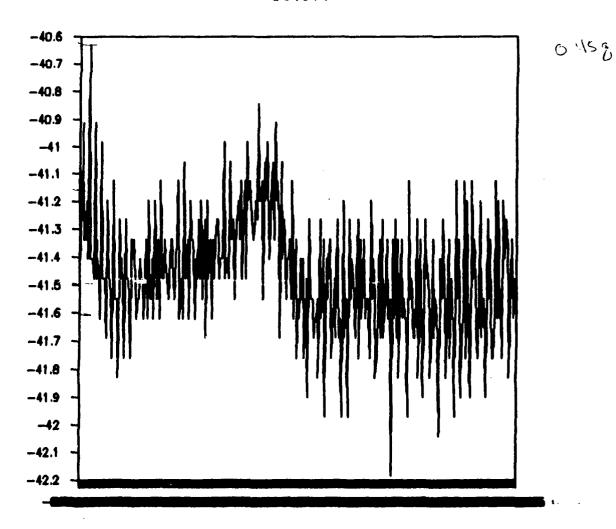


KTP- two strip unwelded blood control

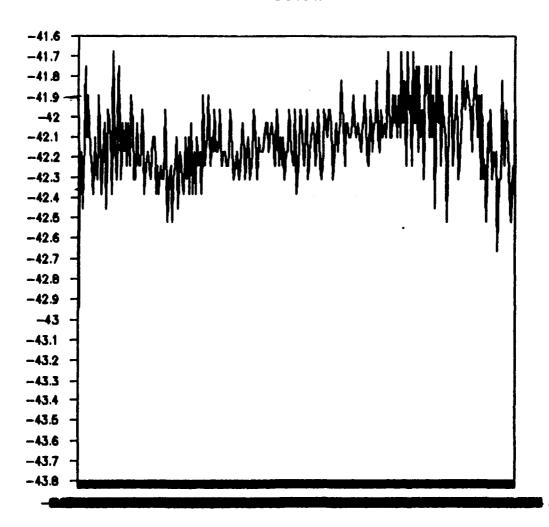
small aperture



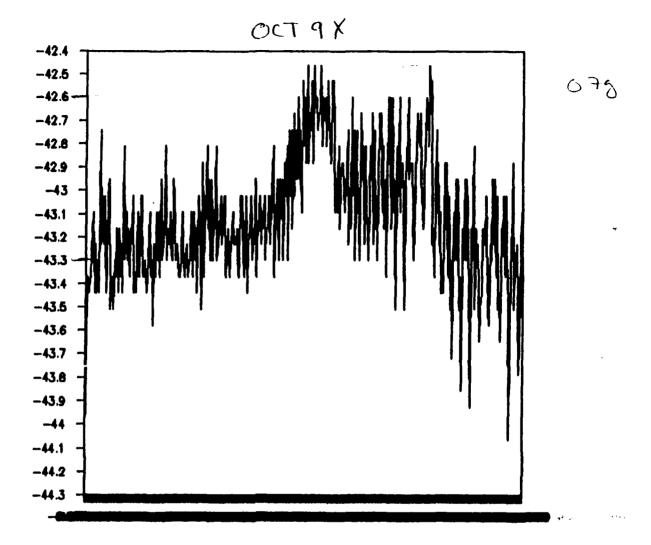
KTP - Blood - 50°C small aperture



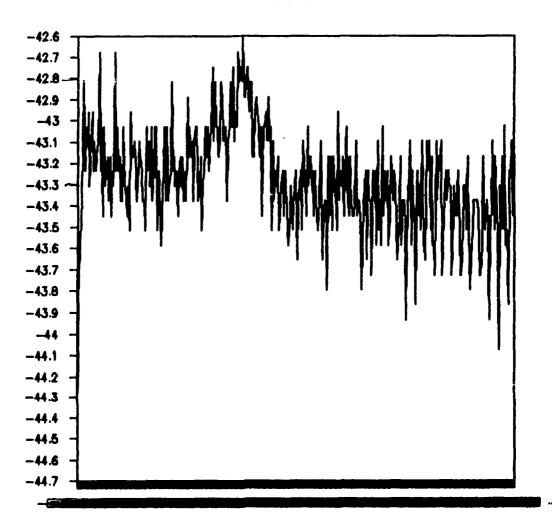
KTP- Blood-60°C small aperture



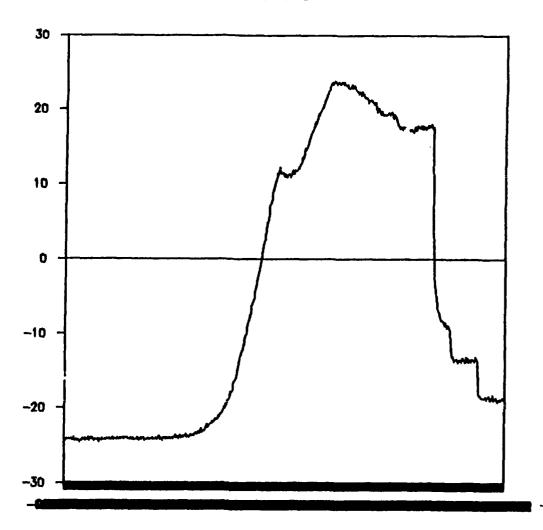
KTP - Blood- 70°C small aperture



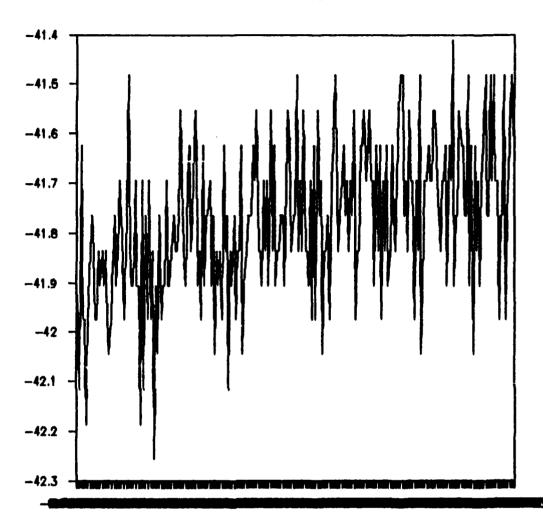
KTP-Blood-80°C small aperture



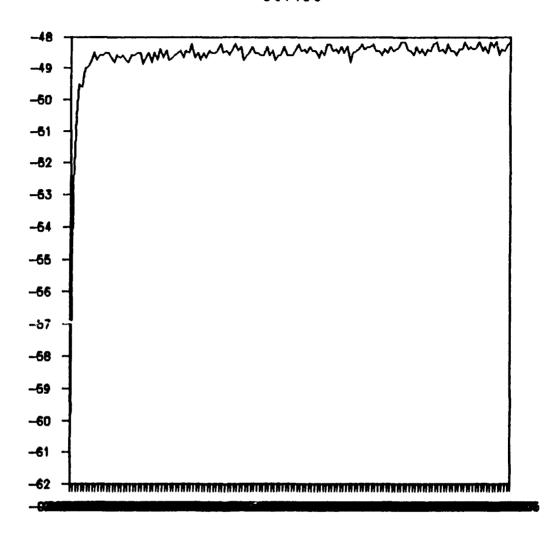
KTP - OCT Blood - 100°C snall aperture



KTP - one strip unwelded control
small aperture

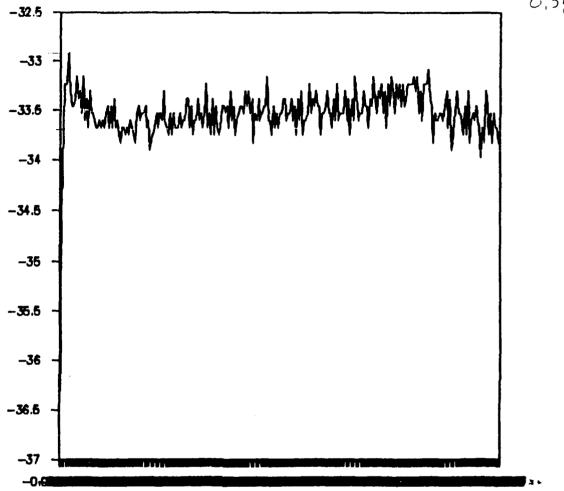


KTP- two stip unwelded control small aperture

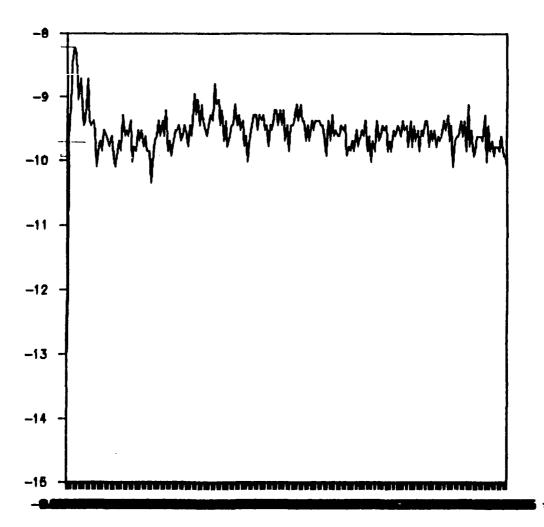


ETP-2 v 1CG unwelded control small aperture

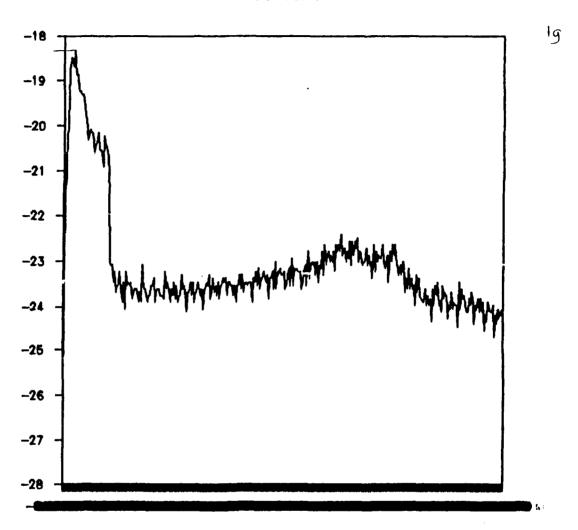




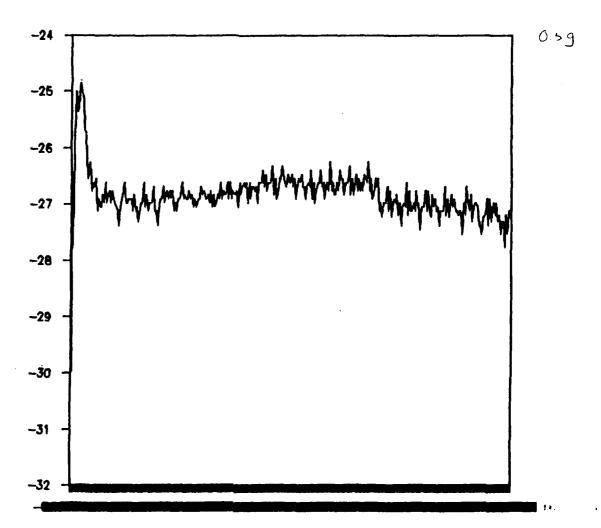
KTP- ax 1(G-50°C small aperture



KTP- 2x 1CG-66°C Small aperture

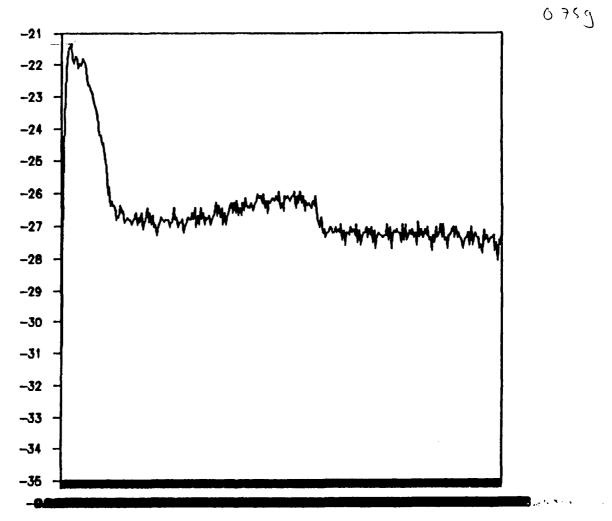


KTP- 2x 1(6-70°C Small aperture

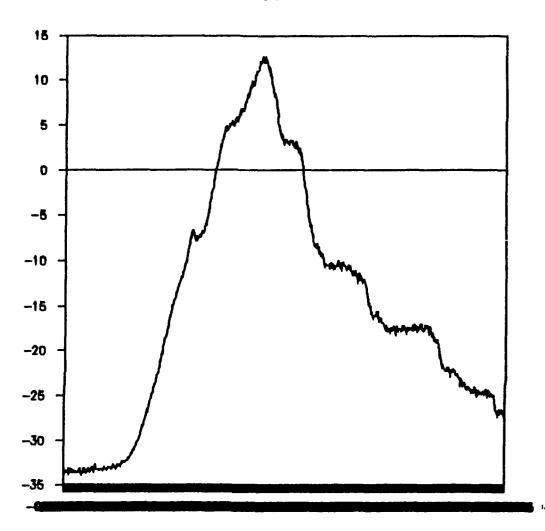


KTP-2×1(G-80°C Small aperture

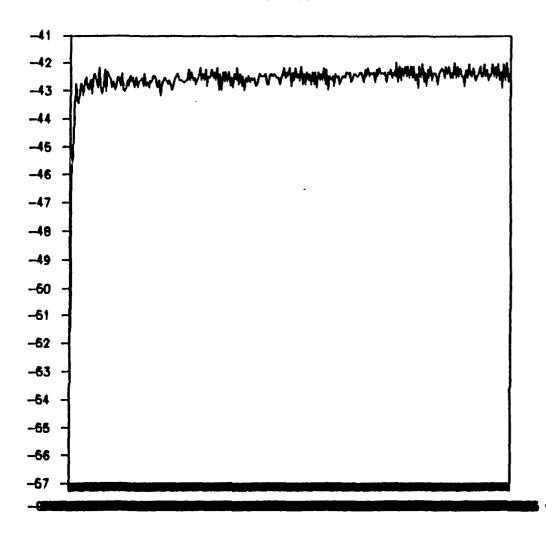




KTP-2x 1CG-100°C Small aperture

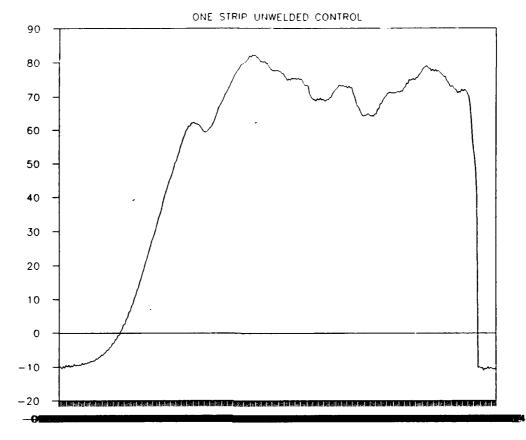


KTP- one stip unwelded control small aperture



KTP- two stip unwelded control Small aperture





All trials for OCT 15,1990

Av<sup>++</sup> Laser

Setting: Current Control

38A, 5.8W

6.7W delivered

Small Aperture

TWO STRIP UNWELDED CONTROL

-10

-11

-12

-13

-14

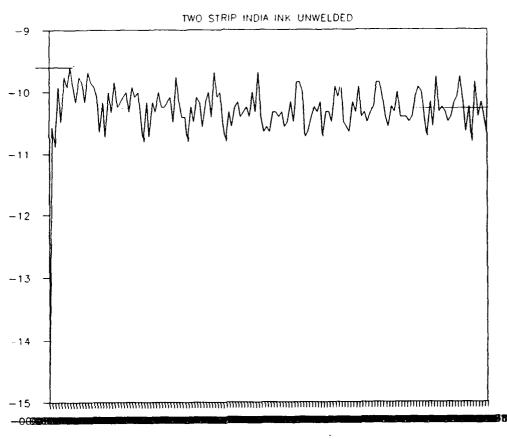
-15

-16

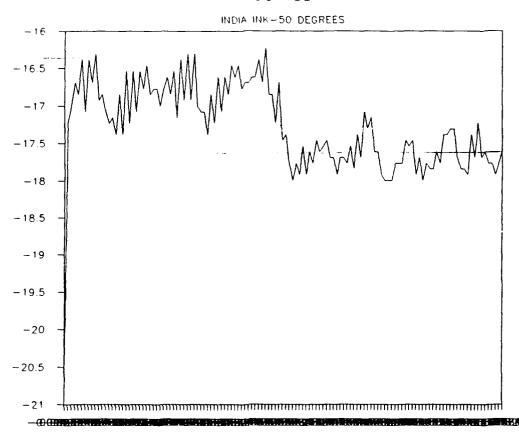
<del>-000004.</del>

OCT15B

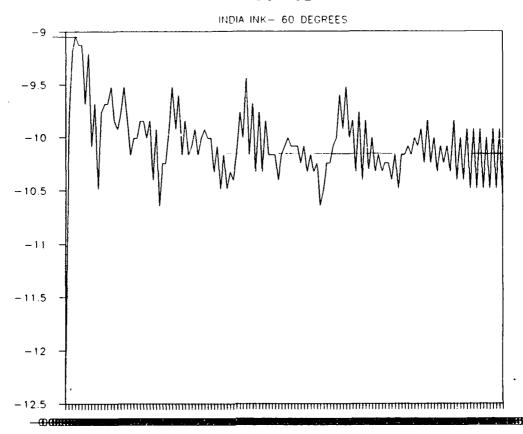
OCT15C



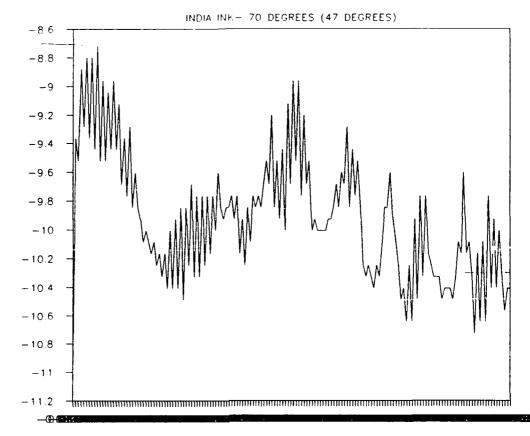




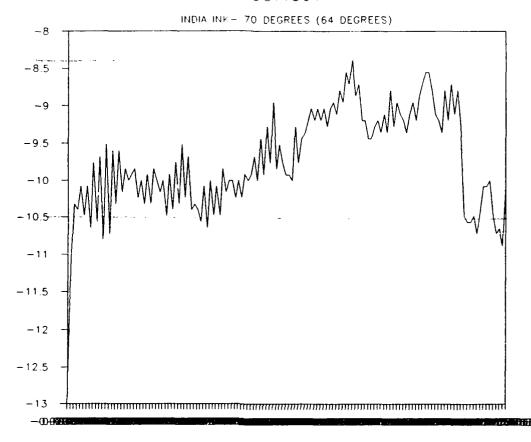
OCT15E



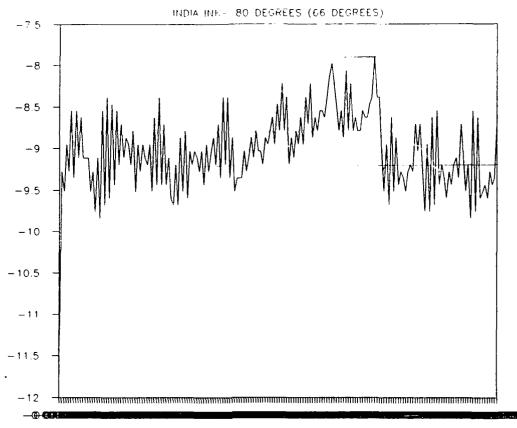
OCT15F1



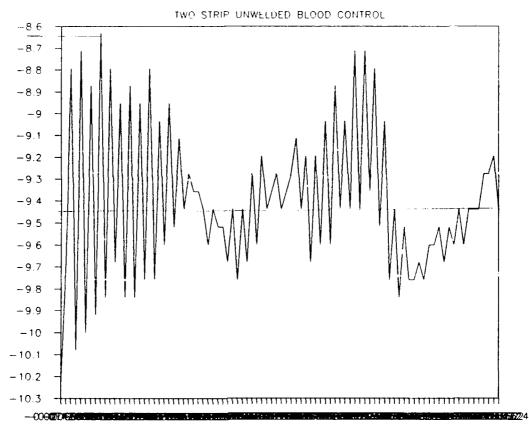
OCT15G1



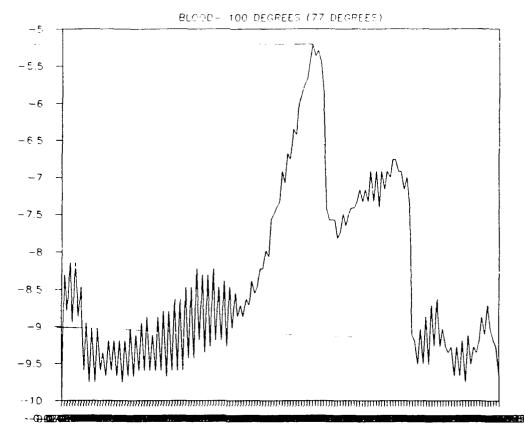
OCT15H1

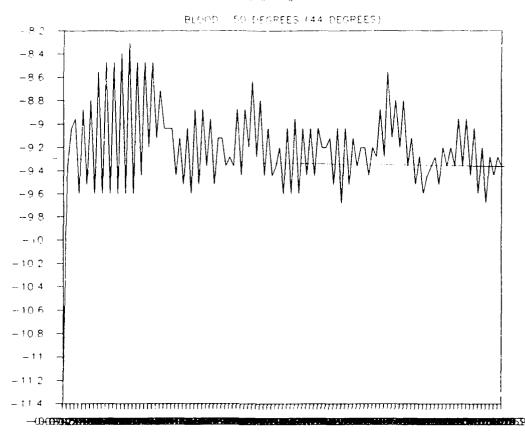


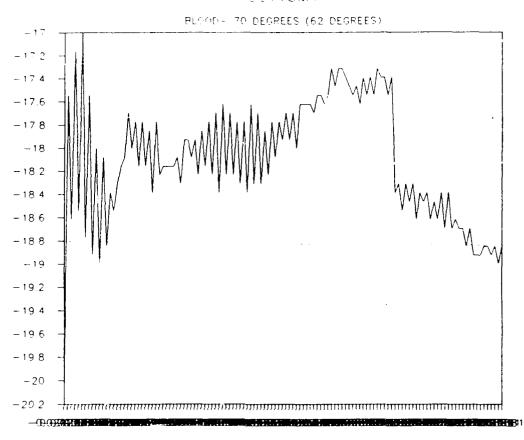
OCT15I



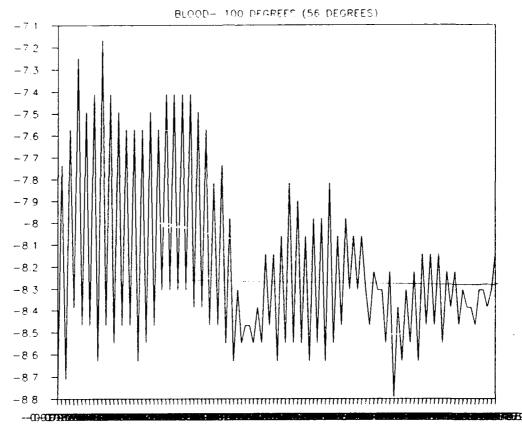
OCT15J1



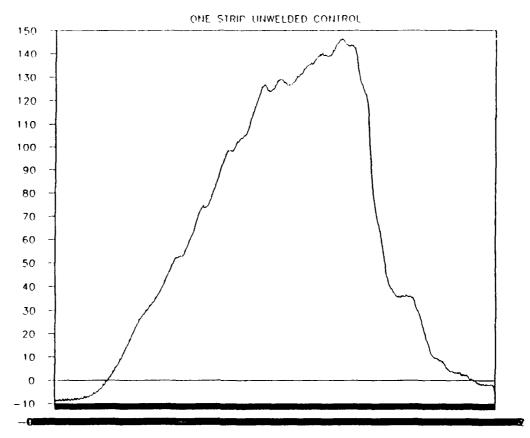




OCT15P1



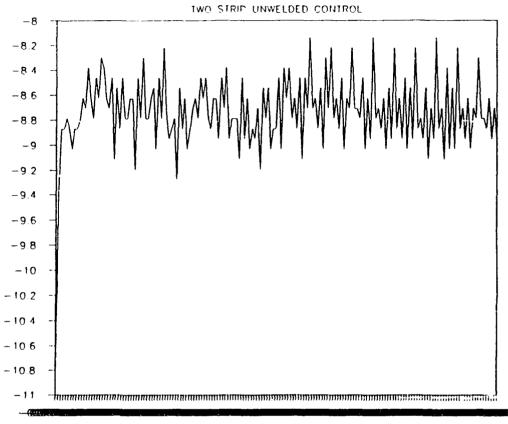




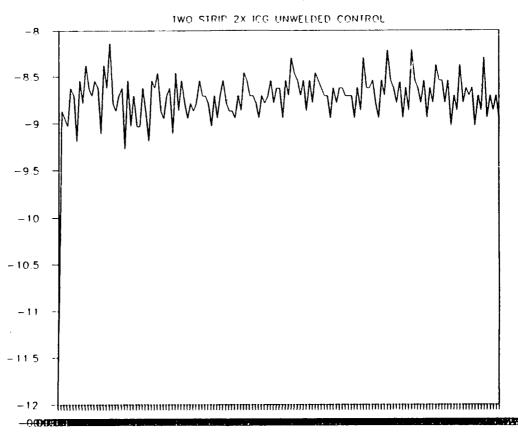
All trials for OCT 16, 1990 Avtt Laser Settrug: Crivent Control 381, 58W

> 0.8W delivered Small Aperture

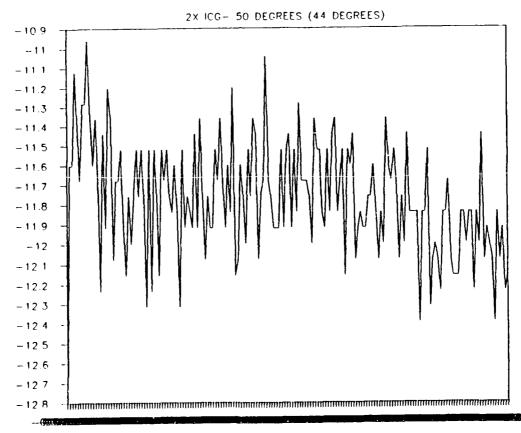
OCT16B



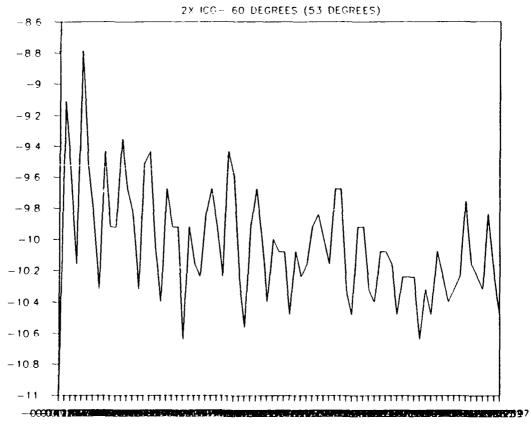
OCT16C



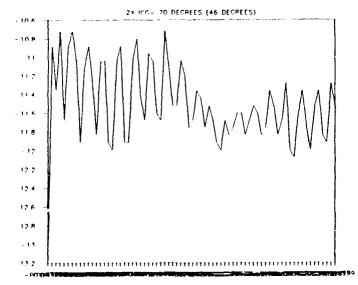
OCT16D1



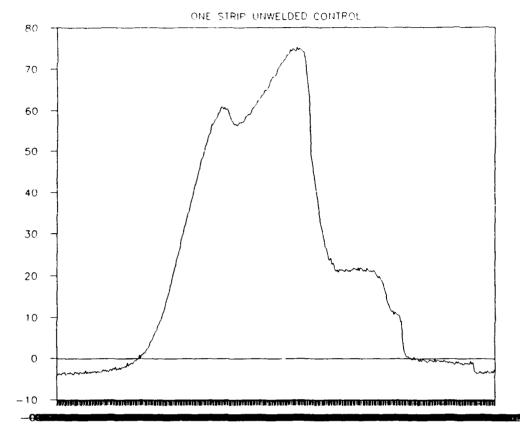
OCT16F









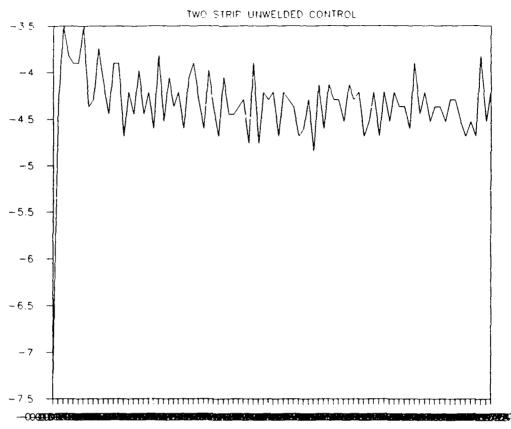


For all ticals on OCT 19,1990 Artt Laser

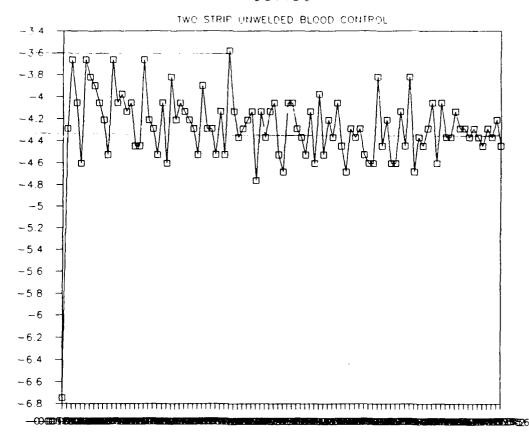
> Settings: Crement Control 32A, 6.0W

> > 0.8 w delivered Small Aperture

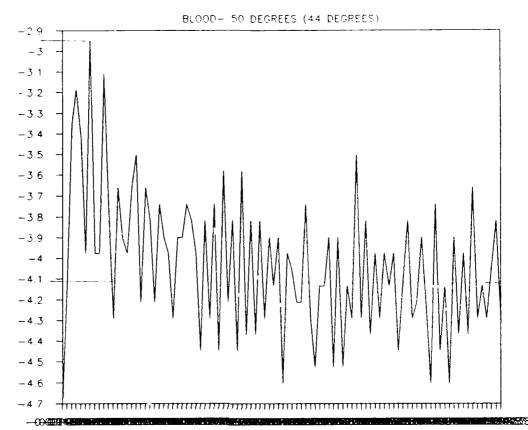
OCT19B

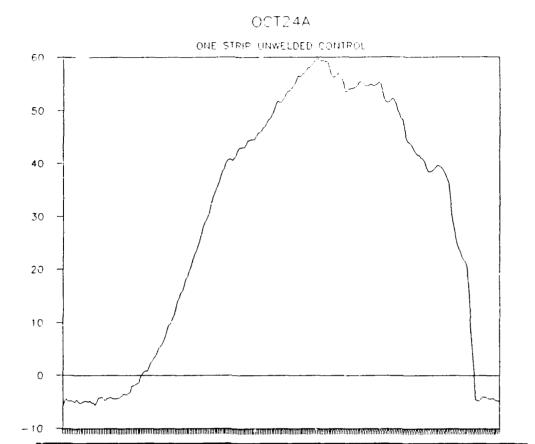


OCT19C



OCT19D1





For all trials on OCT 24,1996

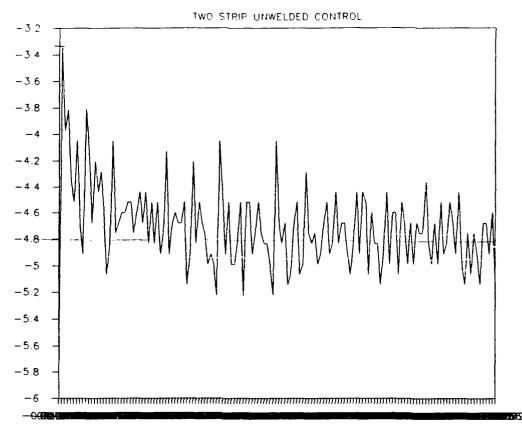
Artt Loser

Setting: Current Control

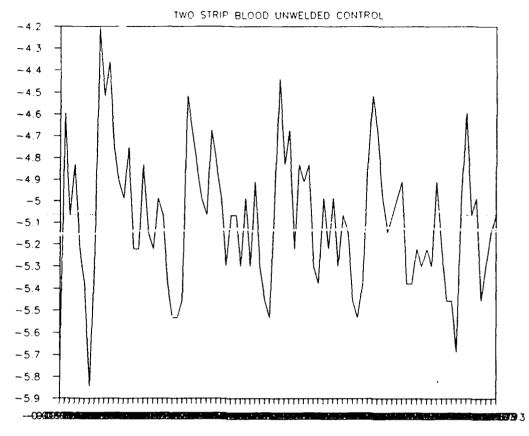
38A, 5.6W

0.7 Washirered Small Aparture

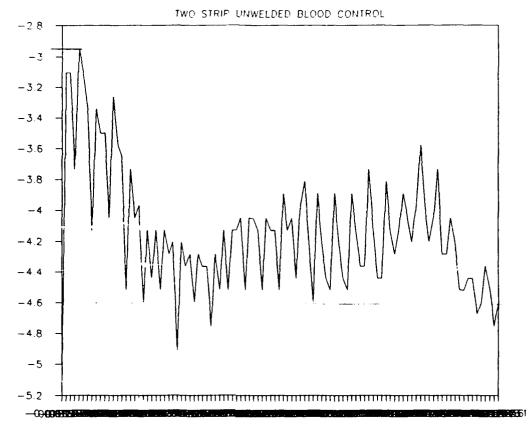
OCT24B



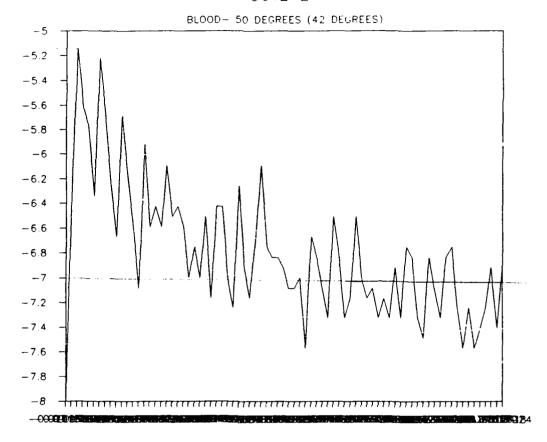
OCT24C



OCT24D

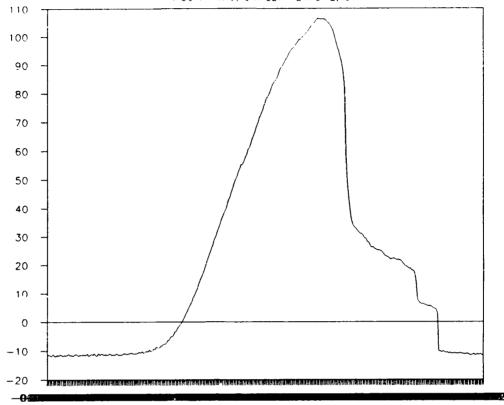


OCT24E1



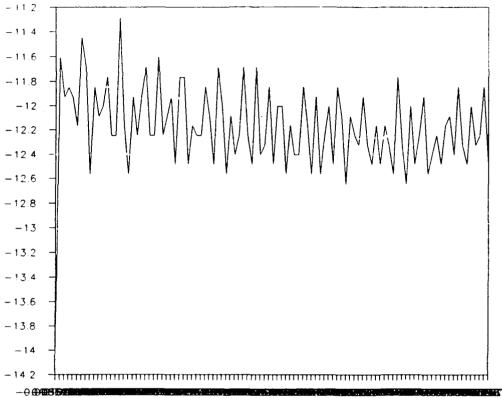
# NOV8A- ONE STRIP CONTROL



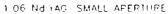


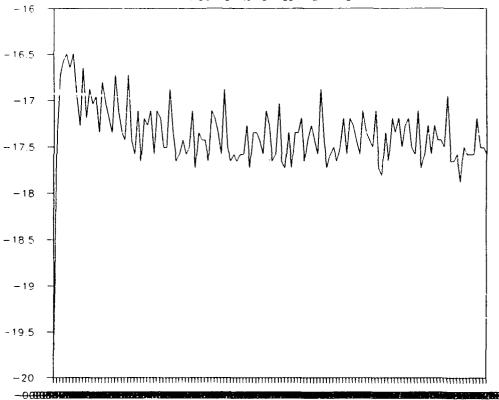
## NOV8C- INDIA INK UNWELDED CONTROL



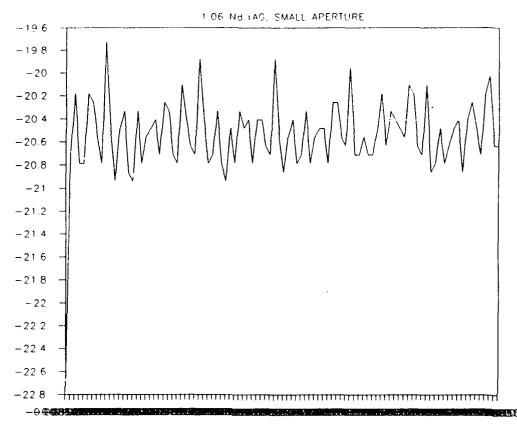


#### NOV8D- INDIA INK CONTROLLED AT 50

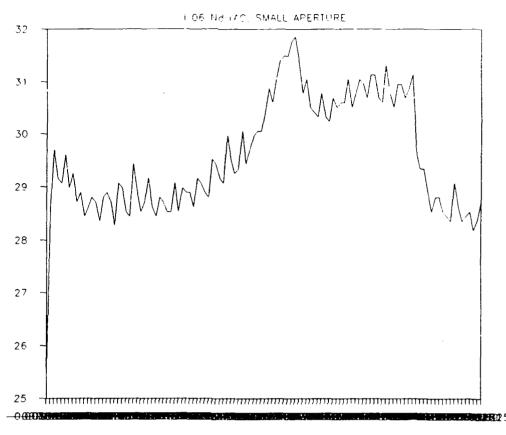




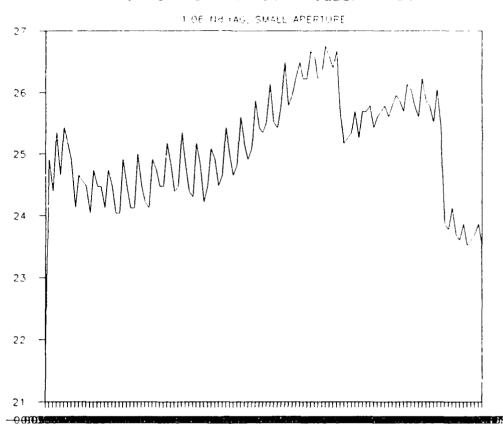
NOVBE- INDIA INK CONTROLLED AT 60



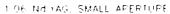
## NOV8F-INDIA INK CONTROLLED AT 70

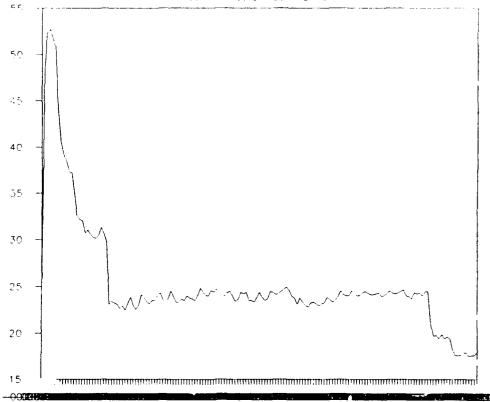


#### NOV8G-INDIA INK CONTROLLED AT 80

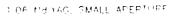


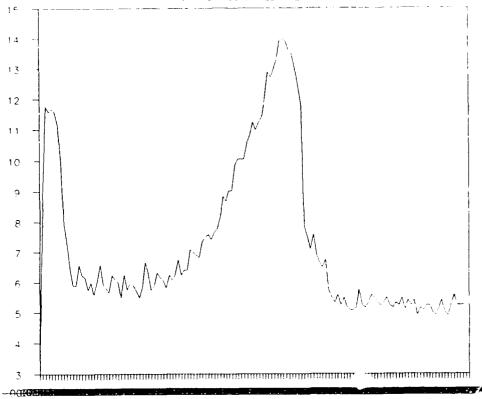
## MOV8H-INDIK INK CONTROLLED AT 100



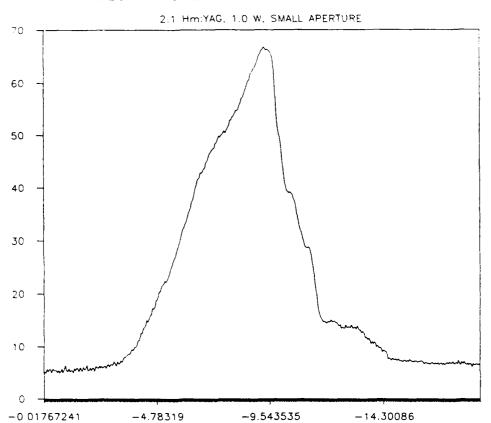


# NOV81-INDIA INK CONTROLLED AT 100

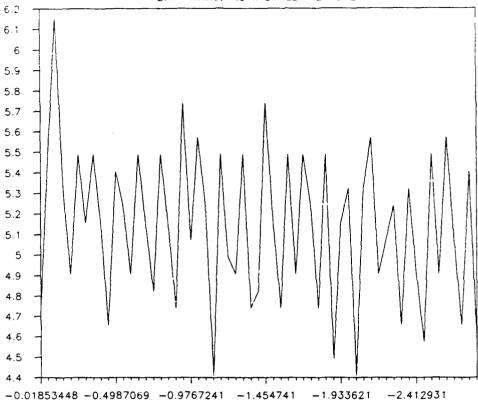




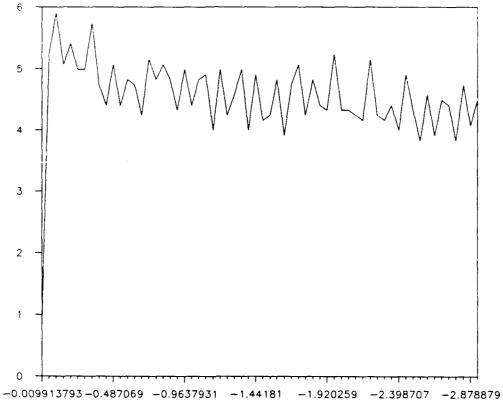
# DEC17A-ONE STRIP UNWELDED CONTROL



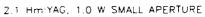
# DEC17B-TWO STRIP UNWELDED CONTROL

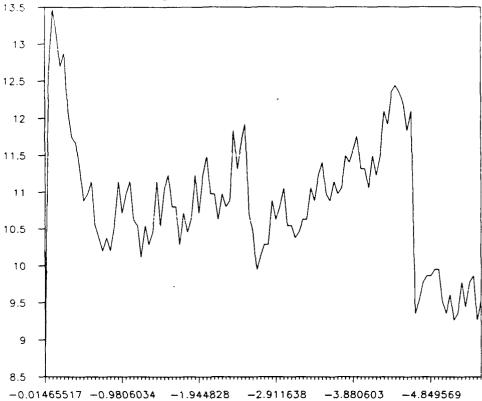


#### DEC17C-TWO STRIP INDIA INK UNWELDED



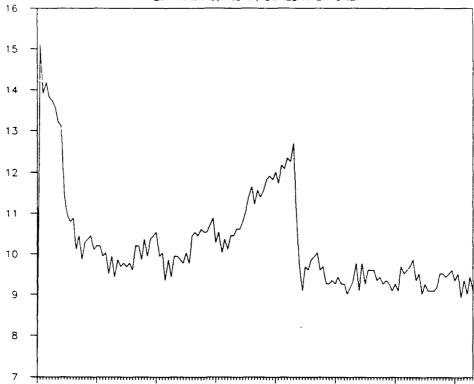
#### DEC17E-INDIA INK CONTROLLED AT 100





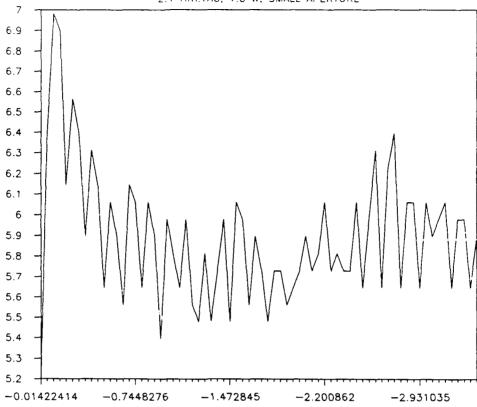
# DEC17F- NO CHROMO SET=100, TC MAX=70



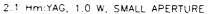


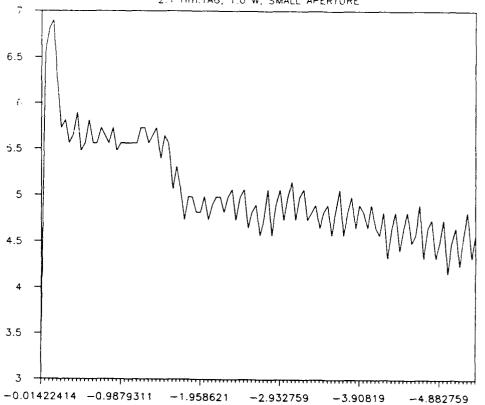
 $-0.00991379 \\ 59.9810344 \\ -1.949138 \\ -2.918104 \\ -3.888793 \\ -4.861207 \\ -5.83319 \\ -6.805173$ 

#### DEC17G-INDIA INK CONTROLLED AT 50, 10s

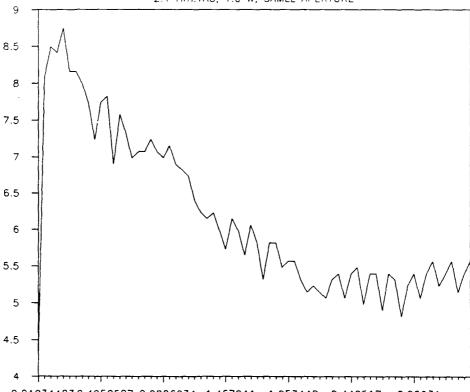


# DEC17H-INDIA INK CONTROLLED AT 60, 10 s



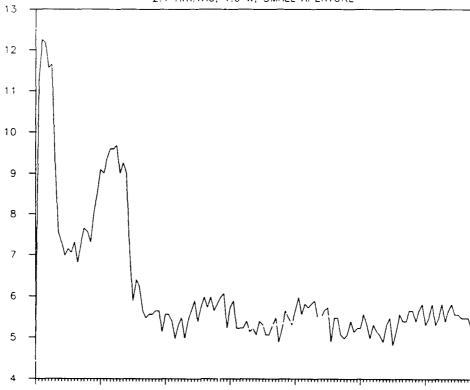


#### DEC17I-INDIA INK CONTROLLED AT 70, 10 s



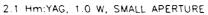
-0.010344830.4952587-0.9806034-1.467241 -1.953448 -2.440517 -2.92931

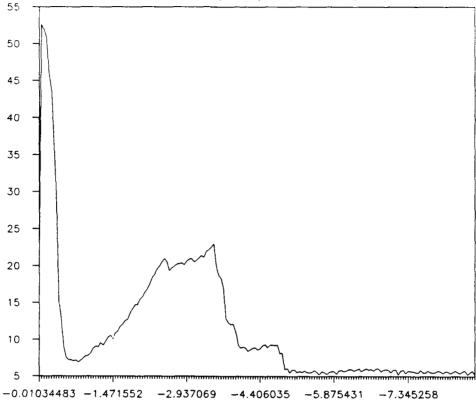
#### DEC17J-INDIA INK CONTROLLED AT 80, 10 s



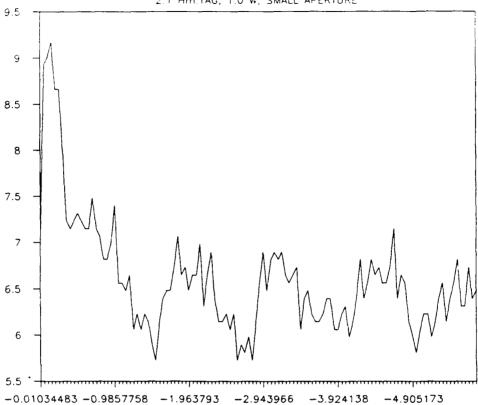
-0.0099137930.9818966 - 1.956034 - 2.932328 - 3.910345 - 4.889224 - 5.868104

# DEC17K-INDIA INK CONTROLLED AT 100, 10s

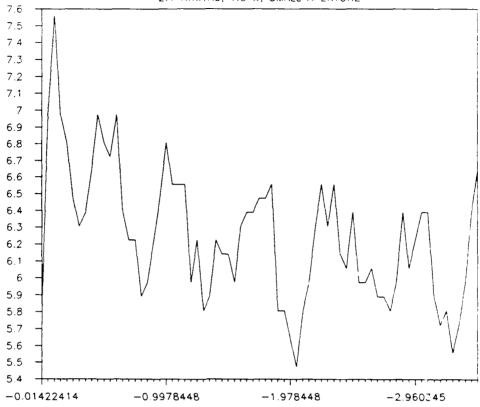




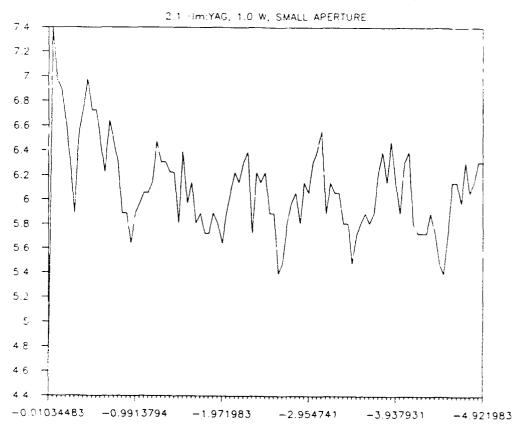
# DEC17L-INDIA INK CONTROLLED AT 100, 10s



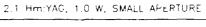
# DEC17M-NO CHROMO, CONTROLLED AT 50, 10s

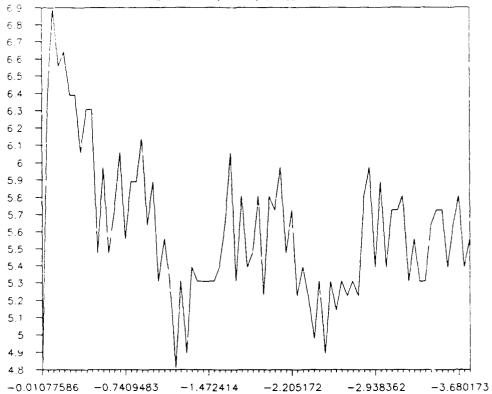


# DEC17N-NO CHROMO CONTROLLED AT 60, 10s

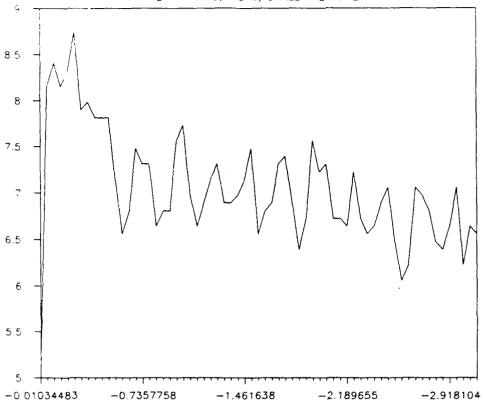


# DEC170-NO CHROMO CONTROLLED AT 70, 10s

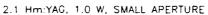


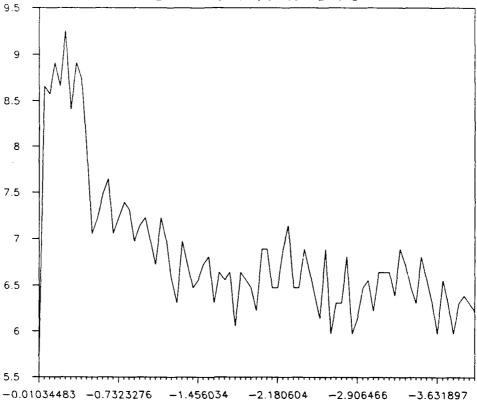


# DEC17P-NO CHROMO CONTROLLED AT 80, 10 s

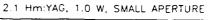


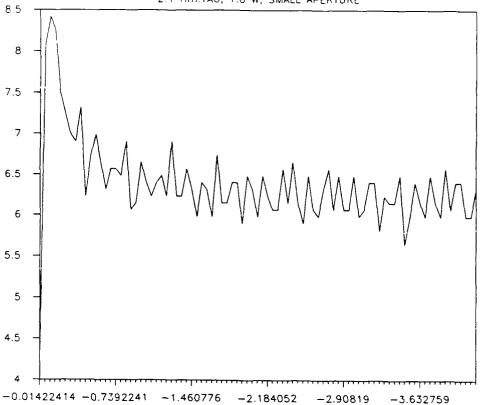
### DEC17Q-NO CHROMO CONTROLLED AT 100, 10s



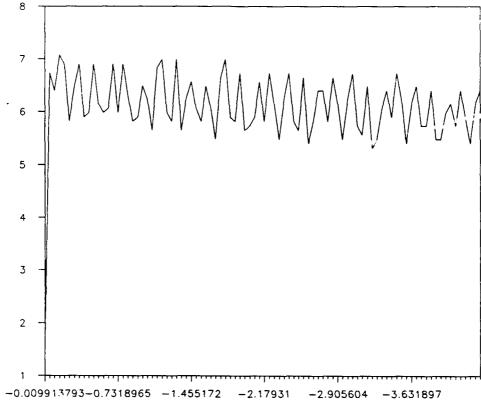


# DEC17R-2X ICG CONTROLLED AT 50, 10s

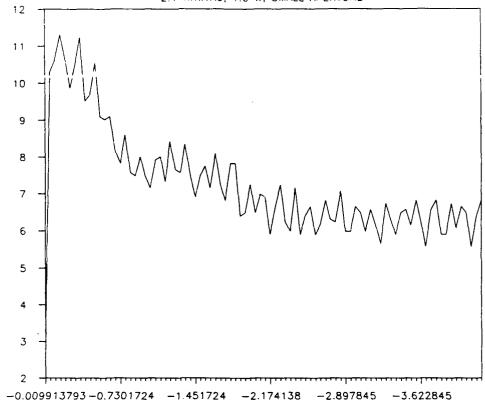




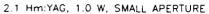
# DEC17S- 2X ICG CONTROLLED AT 60, 10s

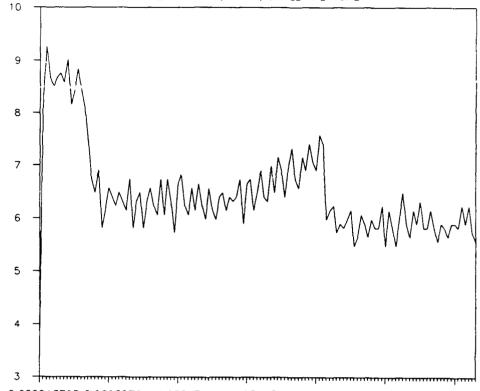


#### DEC17T-2X ICG CONTROLLED AT 70, 10s



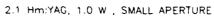
#### DEC17U-2X ICG CONTROLLED AT 80, 10s

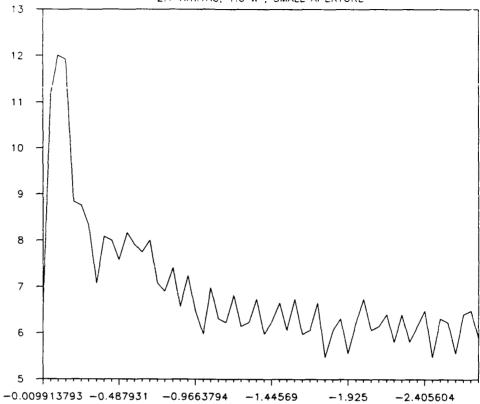




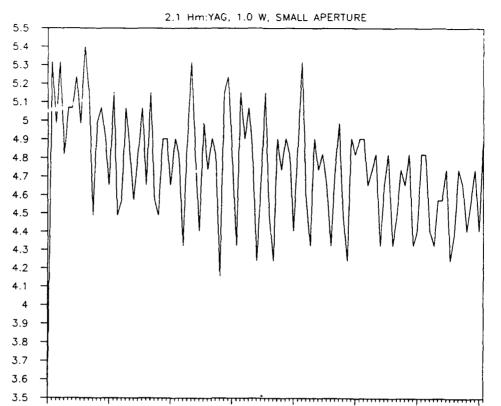
 $-0.009913793 - 0.9698276 \ -1.930172 \ -2.893103 \ -3.856035 \ -4.820259 \ -5.784914$ 

# DEC17V-2X ICG SET AT 100, TC MAX=90,



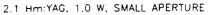


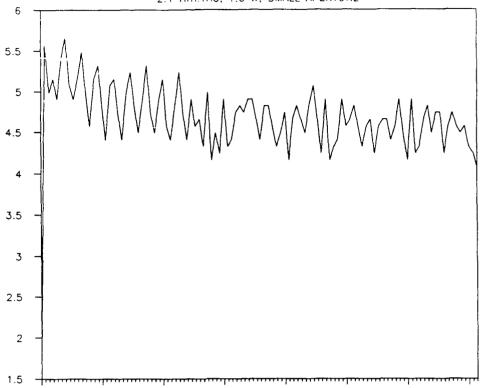
# DEC17W- 2X ICG UNWELDED CONTROL



 $-0.0103448\overline{\smash)0.7288793} -1.447414 -2.168104 -2.889224 -3.611207 -4.332759 -5.055604$ 

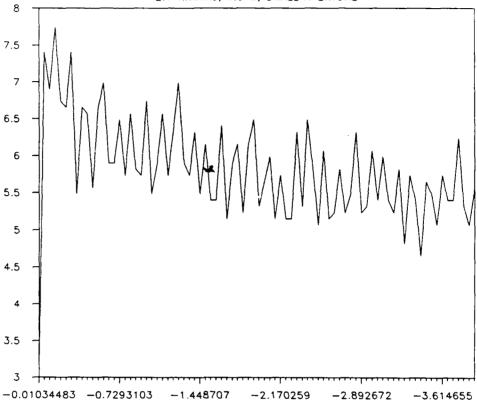
# DEC17X-BLOOD UNWELDED CONTROL



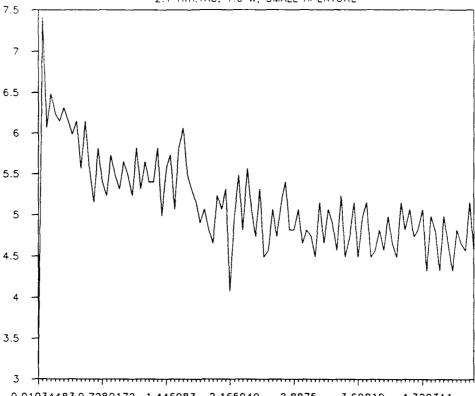


 $-0.00991379 \cdot 0.7306035 - 1.452586 - 2.174138 - 2.896983 - 3.621121 - 4.344828 - 5.069828 - 2.174138 - 2.896983 - 3.621121 - 4.344828 - 5.069828 - 2.174138 - 2.896983 - 3.621121 - 4.344828 - 5.069828 - 3.621121 - 4.348828 - 5.069828 - 3.621121 - 4.348828 - 5.069828 - 3.621121 - 4.348828 - 5.069828 - 3.621121 - 4.348828 - 5.069$ 

#### DEC17Y- BLOOD CONTROLLED AT 50, 10 s

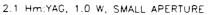


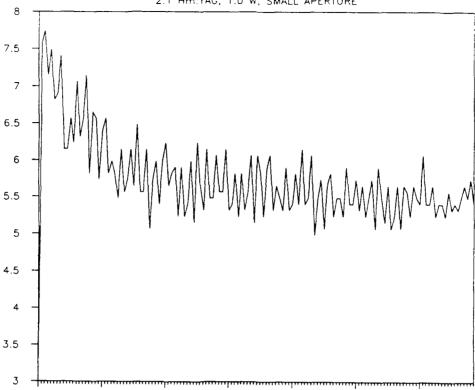
#### DEC17Z-BLOOD CONTROLLED AT 60, 10s



-0.010344830.7280172 - 1.446983 - 2.165949 - 2.8875 - 3.60819 - 4.329311

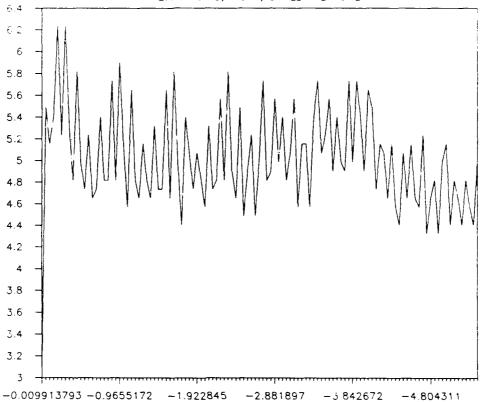
#### DEC17AA- BLOOD CONTROLLED AT 70, 10s



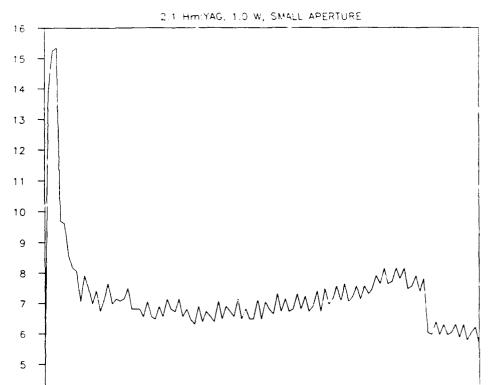


-0.0099137930.9672414 - 1.926724 - 2.888362 - 3.850862 - 4.813793 - 5.777156

# DEC17AB- BLOOD CONTROLLED AT 80, 10 s



#### DEC17AC-BLOOD CONTROLLED AT 100, 10 s

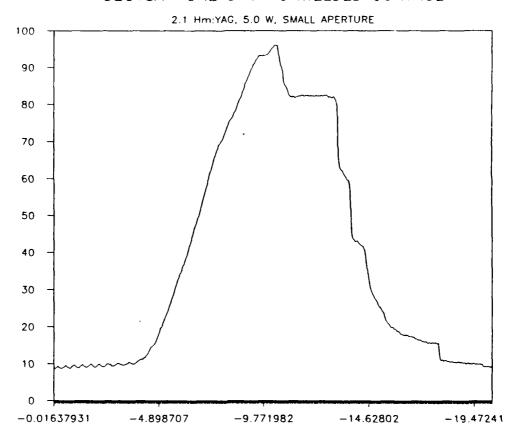


--2.876293 --3.873621

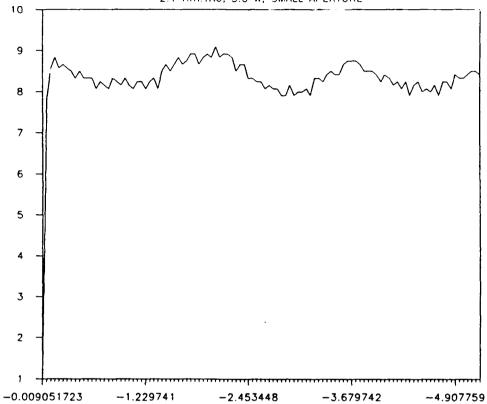
-4.792672

-0.01034483 -0.9637931 -1.918535

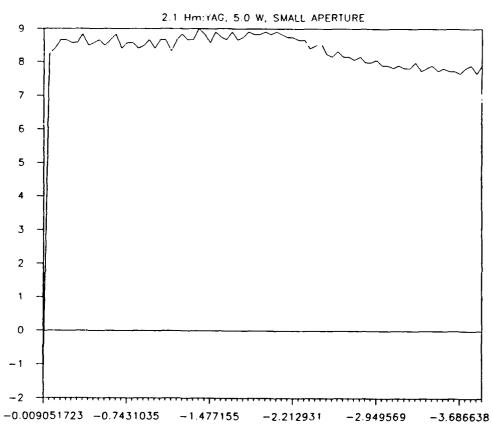
# DEC18A- ONE STRIP UNWELDED CONTROL



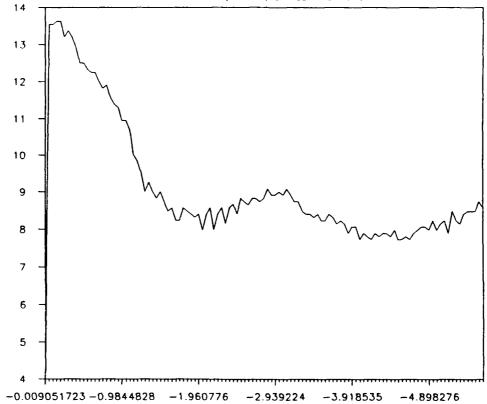
# DEC18B-TWO STRIP UNWELDED CONTROL



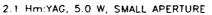
# DEC18C- INDIA INK UNWELDED CONTROL

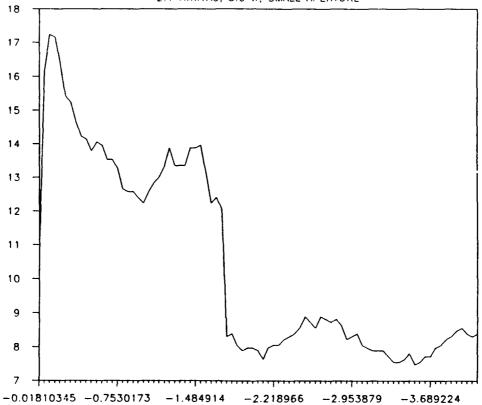


# DEC18D-INDIA INK CONTROLLED AT 50, 10 s

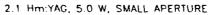


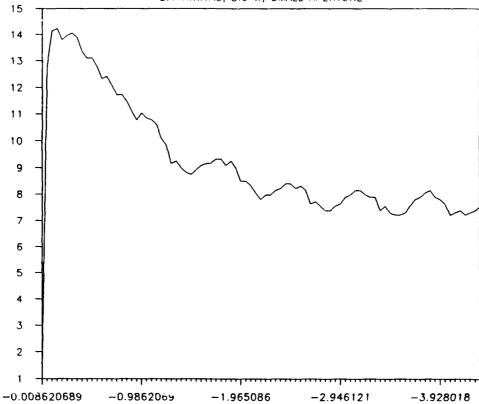
# DEC18E1-INDIA INK CONTROLLED AT 60, 10s





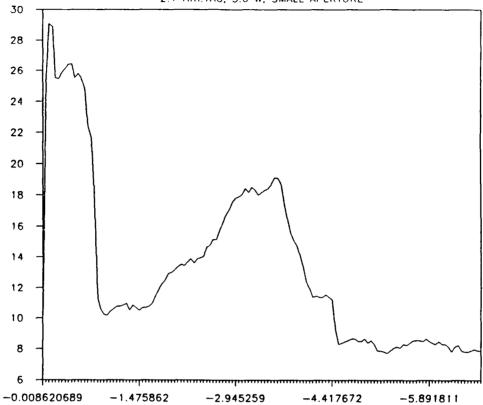
#### DEC18F-INDIA INK CONTROLLED AT 70, 10s



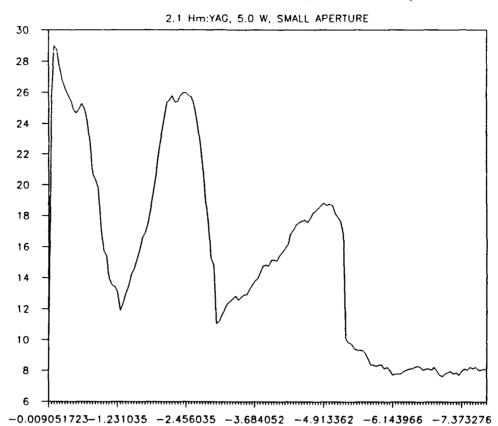


DEC18G- INDIA INK CONTROLLED AT 80, 10s

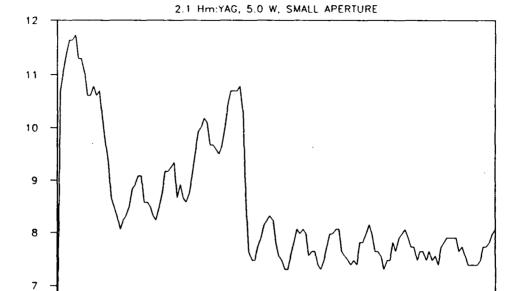




#### DEC18H-INDIA INK CONTROLLED AT 100, 10s



### DEC18I- NO CHROM CONTROLLED AT 50, 10s



-2.958621

-4.4375

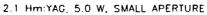
-5.918535

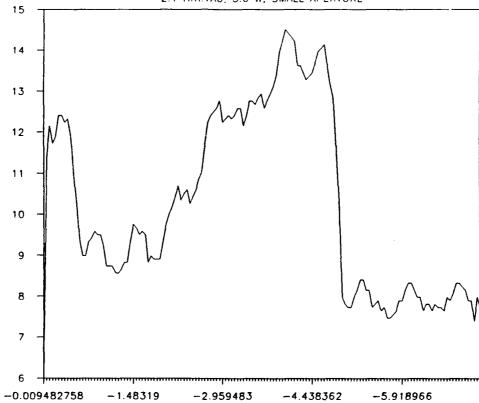
6

-0.009482758

-1.482759

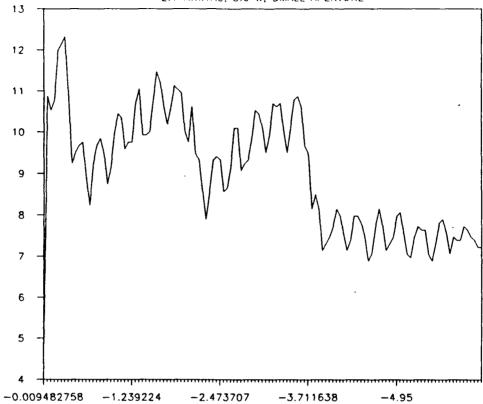
#### DEC18J-NO CHROMO CONTROLLED AT 60, 10s



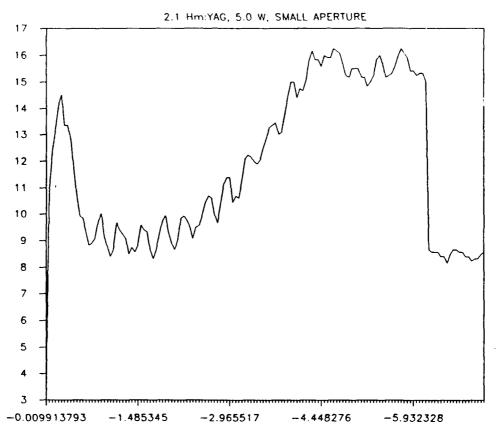


## DEC18K- NO CHROMO CONTROLLED AT 60, 10s

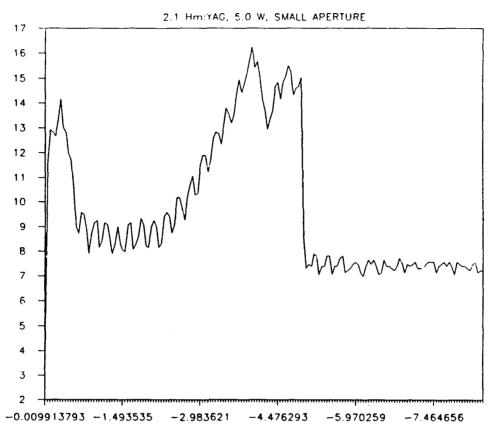
2.1 Hm:YAG, 5.0 W, SMALL APERTURE



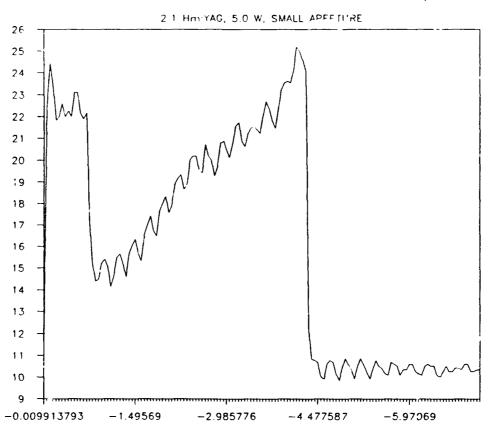
## DEC18L-NO CHROMO CONTROLLED AT 70, 10 s



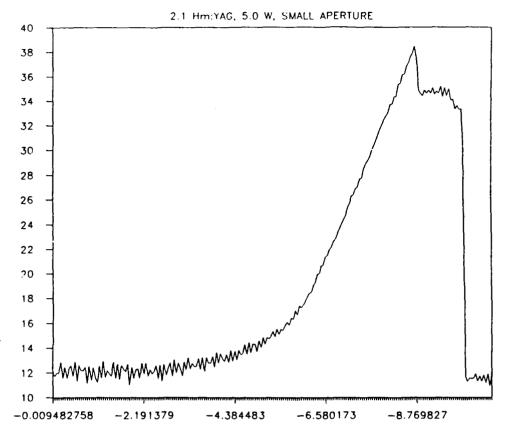
### DEC18N-NO CHROMO-CONTROLLED AT 80, 10 s



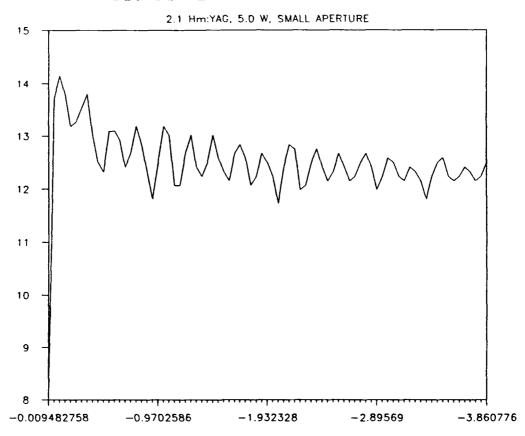
### DEC180- NO CHROMO CONTROLLED AT 100,10s



DEC18P-NO CHROMO CONTROLLED AT 100, 10s

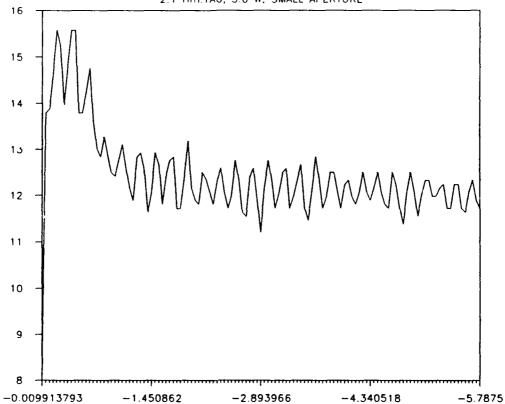


#### DEC18Q- 2X ICG UNWELDED CONTROL



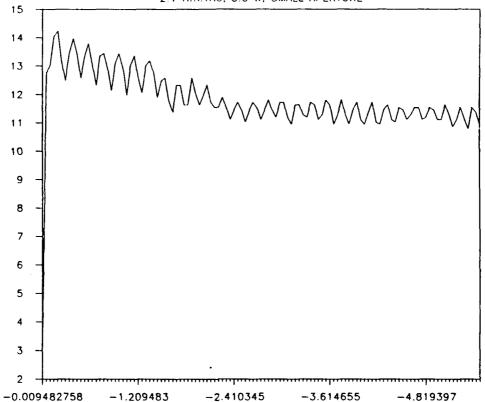
### DEC18R- 2X ICG CONTROLLED AT 50,10 s

2.1 Hm:YAG, 5.0 W, SMALL APERTURE

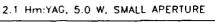


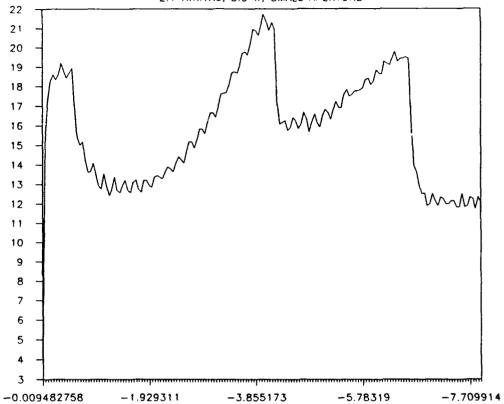
#### DEC18S- 2X ICG CONTROLLED AT 60, 10 s

2.1 Hm:YAG, 5.0 W, SMALL APERTURE

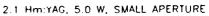


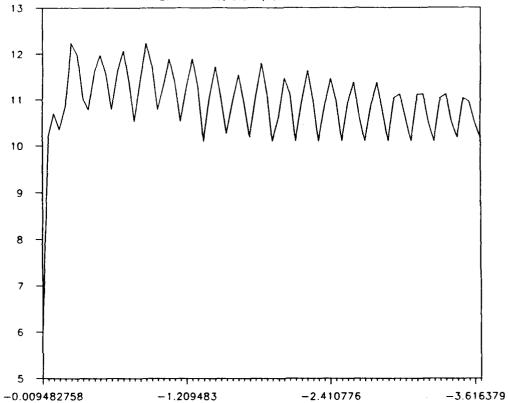
DEC18T- 2X ICG CONTROLLED AT 70, 10 s



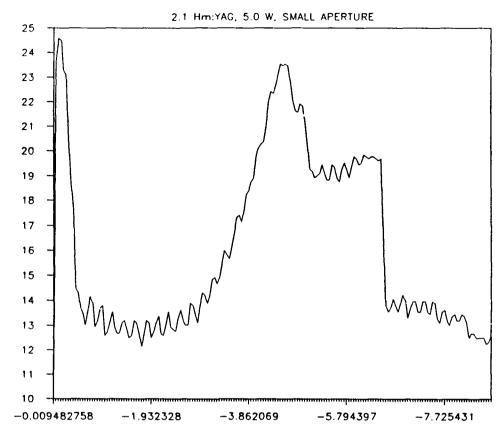


DEC18U- 2X ICG CONTROLLED AT 80, 10s

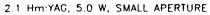


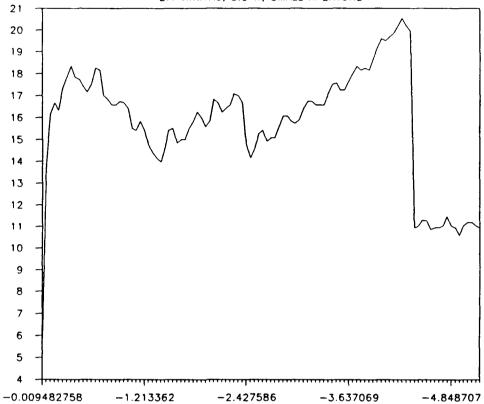


### DEC18V- 2X CIG CONTROLLED AT 100, 10s



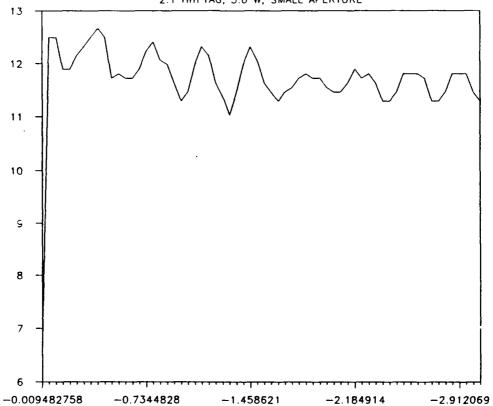
## DEC18W1- 2X ICG CONTROLLED AT 80, 10s



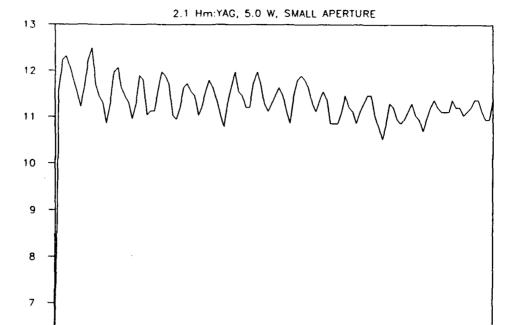


## DEC18X- BLOOD UNWELDED CONTROL

2.1 Hm:YAG, 5.0 W, SMALL APERTURE



DEC18Y- BLOOD CONTROLLED AT 50, 10 s



-2.908021

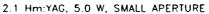
-4.362932

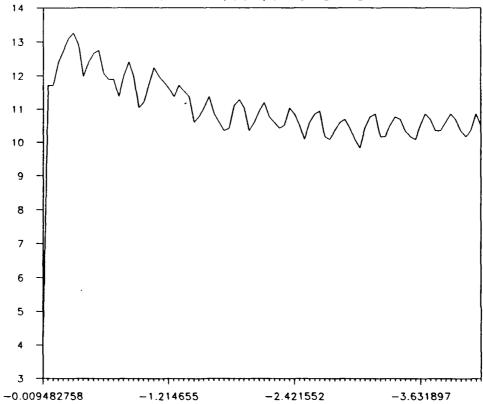
6

-0.009482758

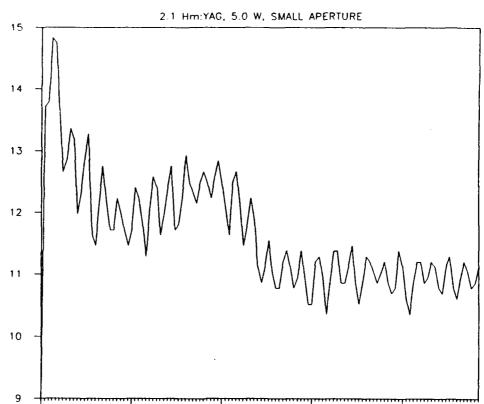
-1.456897

DEC18Z- BLOOD CONTROLLED AT 50, 10 s





### DEC18AA- BLOOD CONTROLLED AT 60, 10s



-2.419397

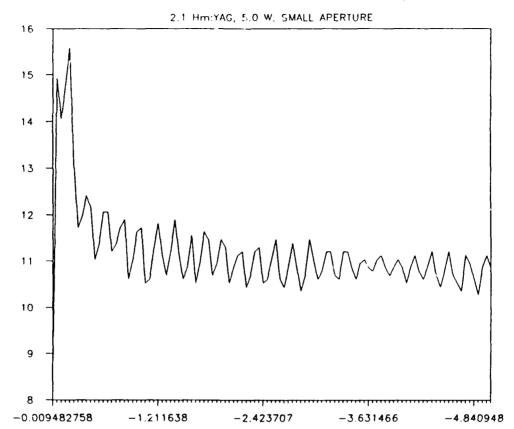
-3.629741

-4.839656

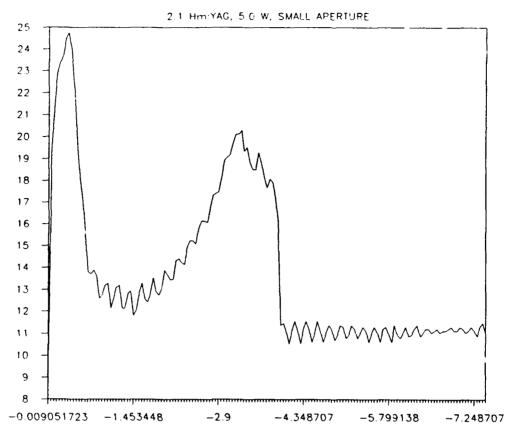
-0.009482758

-1.212931

#### DEC18AB- BLOOD CONTROLLED AT 70, 10 s

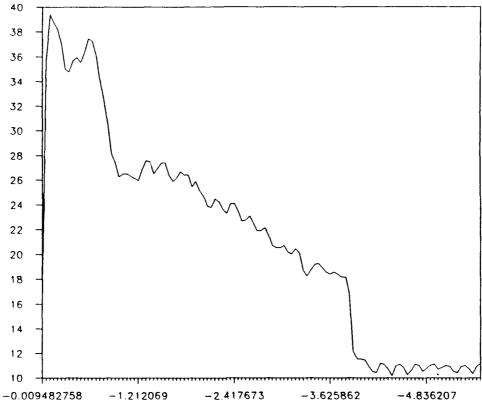


### DEC18AC - BLOOD CONTROLLED AT 80, 10 s

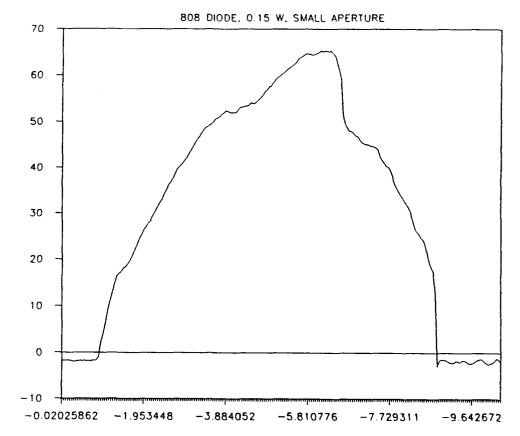


### DEC18AD- BLOOD CONTROLLED AT 100, 10 s

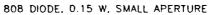


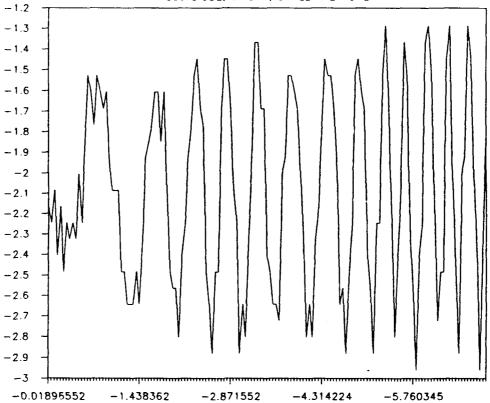


# DEC19A- ONE STRIP UNWELDED CONTROL

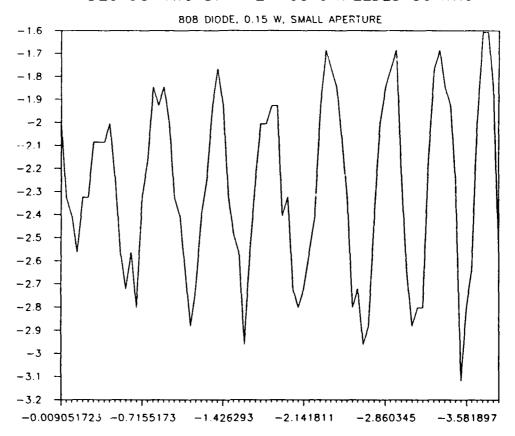


### DEC198- TWO STRIP UNWELDED CONTROL



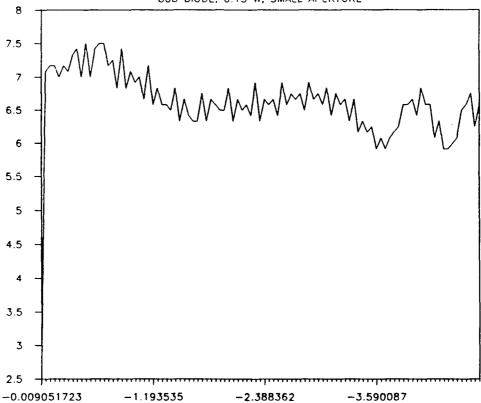


### DEC19C-TWO STRIP 2X ICG UNWELDED CONTRO



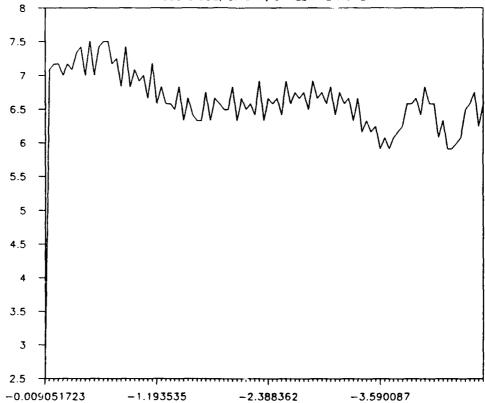
#### DEC19D-2X ICG SET=50, TC MAX=20

808 DIODE, 0.15 W, SMALL APERTURE



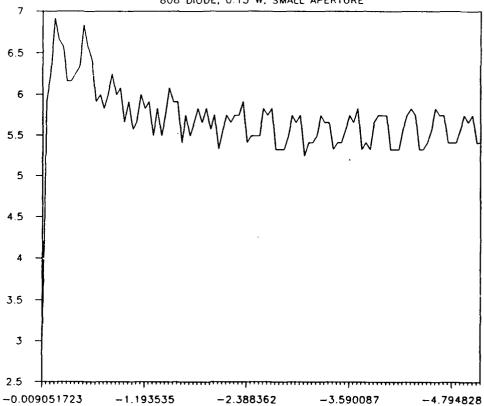
## DEC19D-2X ICG SET=50, TC MAX=20

808 DIODE, 0.15 W, SMALL APERTURE

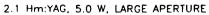


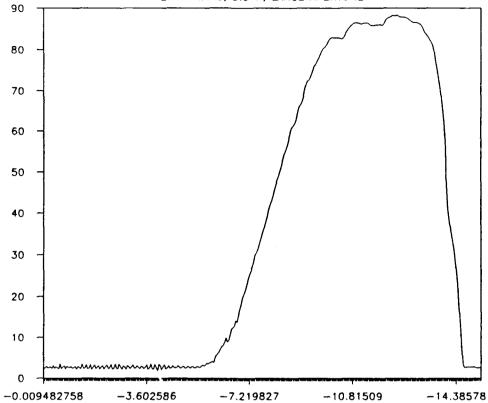
## DEC19E-2X ICG SET=100, TC MAX=20



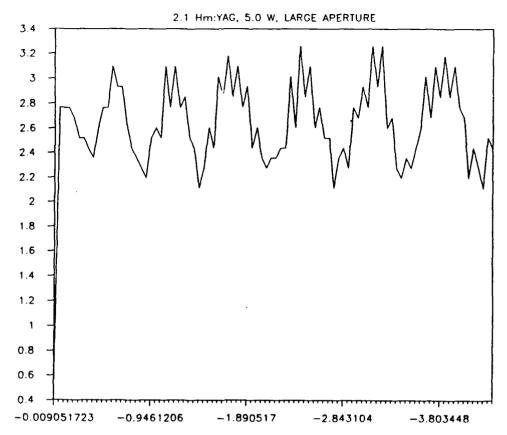


#### DEC19F-ONE STRIP UNWELDED CONTROL

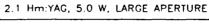


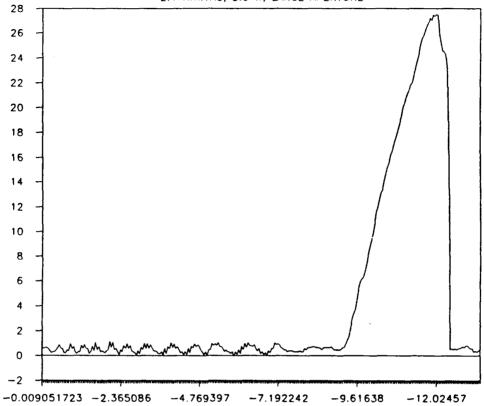


## DEC19G-TWO STRIP UNWELDED CONTROL

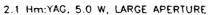


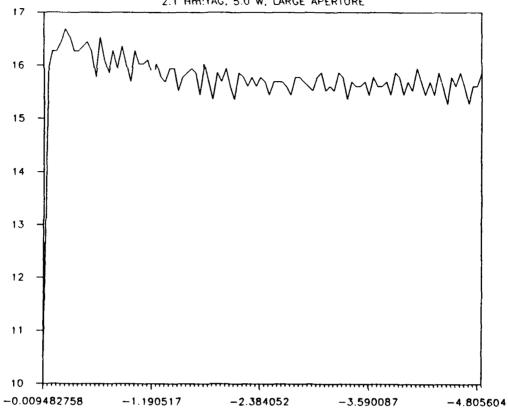
### DEC19H-NO CHROMO CONTROLLED AT 50, 10 s





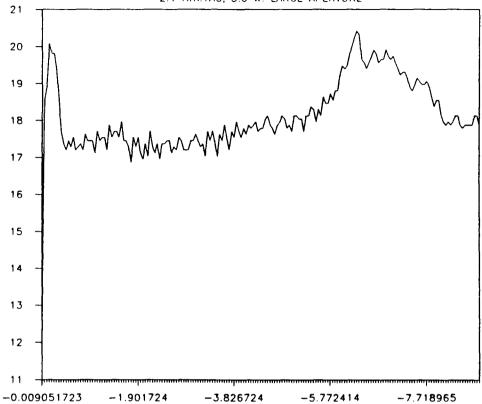
### DEC191-NO CHROMO CONTROLLED AT 60, 10s



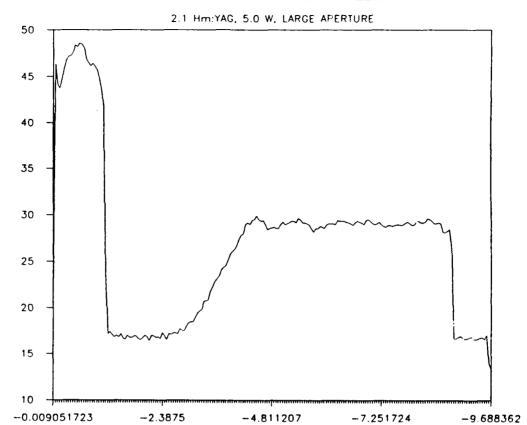


#### DEC19J-NO CHROMO CONTROLLED AT 70, 10 s

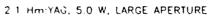
2.1 Hm:YAG, 5.0 W. LARGE APERTURE

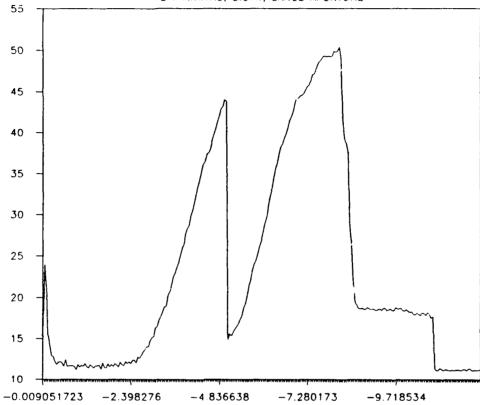


### DEC19K- NO CHROMO CONTROLLED AT 80

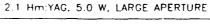


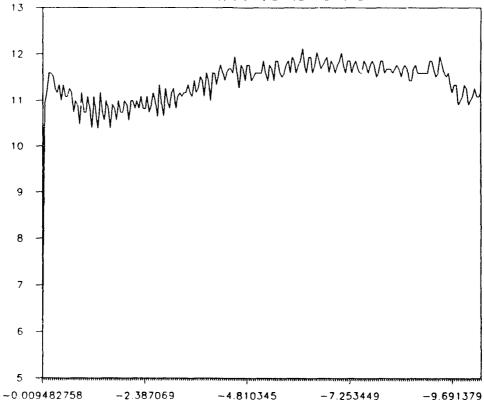
# DEC19L-NO CHROMO CONTROLLED AT 100, 10s





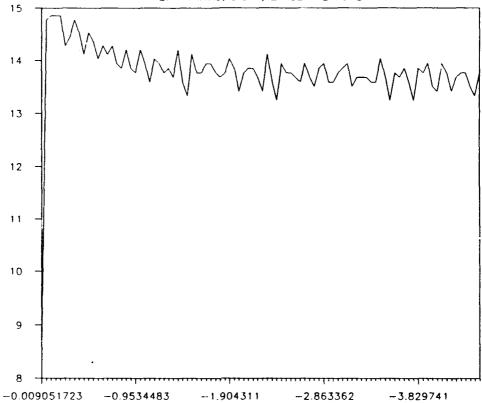
#### DEC19M- NO CHROMO CONTROLLED AT 50, 10s



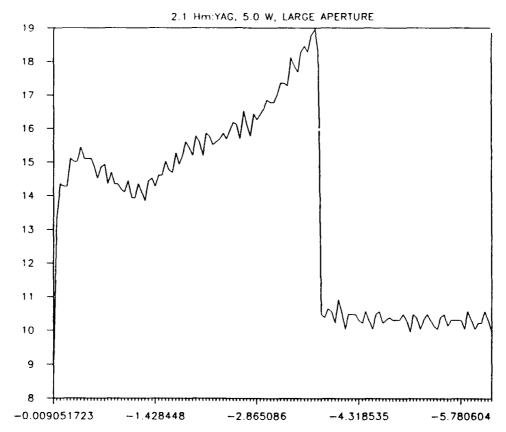


### DEC19N- INDIA INK UNWELDED CONTROL

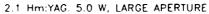
2.1 Hm:YAG, 5.0 W, LARGE APERTURE

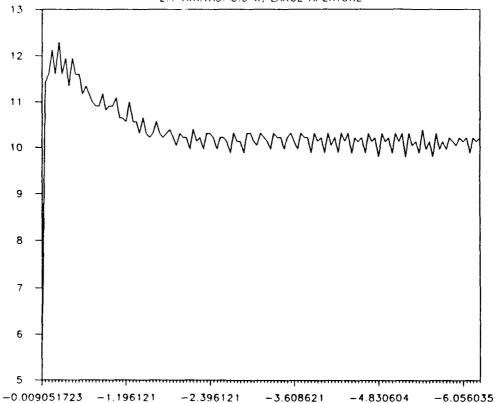


# DEC190-INDIA INK CONTROLLED AT 50, 10s

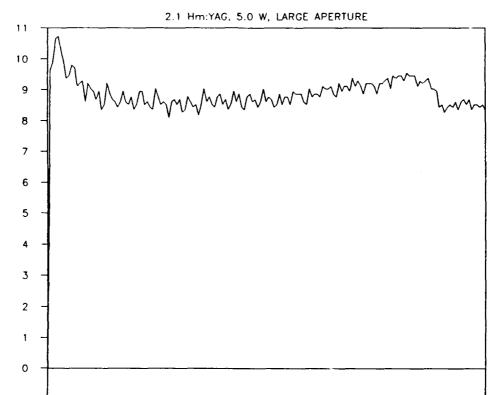


DEC19P1-INDIA INK CONTROLLED AT 50, 'Os





# DEC19Q-INDIA INK CONTROLLED AT 60, 10s



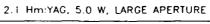
-4.848276

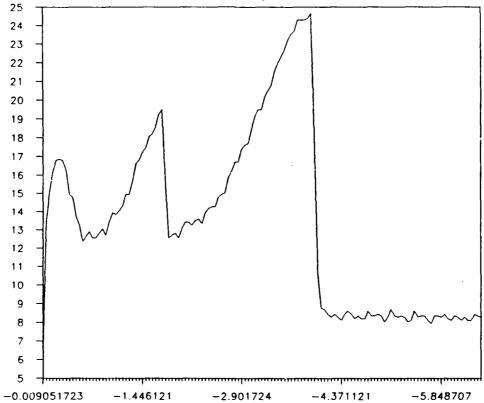
-7.303018

-2.407328

-0.009482758

DEC19R- INDIA INK CONTROLLED AT 70, 10s

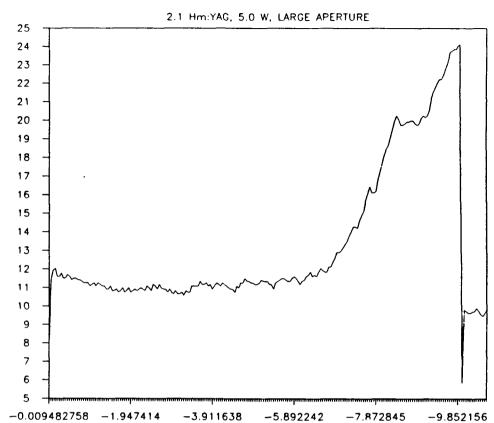




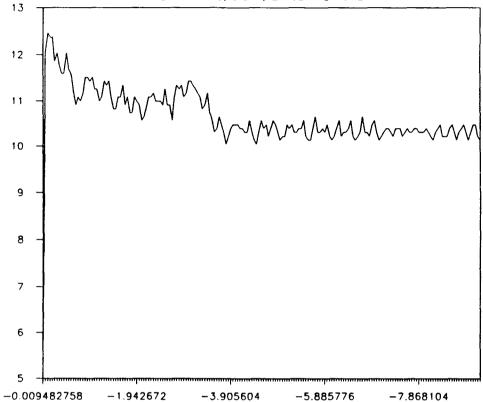
-2.901724

-1.446121

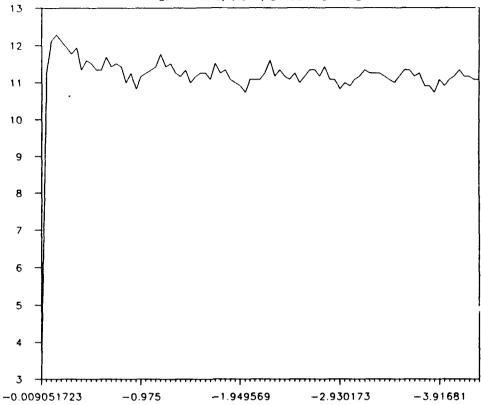
# DEC19S- INDIA INK CONTROLLED AT 80, 10s



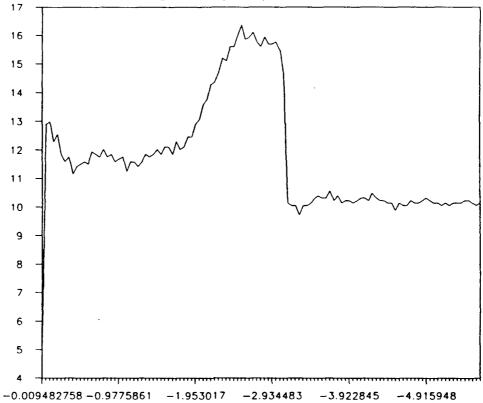
### DEC19T-INDIA INK CONTROLLED AT 100, 10s



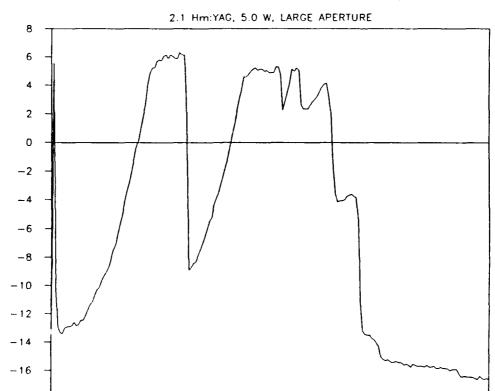
# DEC19U-2X ICG UNWELDED CONTROL



DEC19V- 2X ICG CONTROLLED AT 50, 10s



DEC19W-2X ICG CONTROLLED AT 60, 10s



-4.951293

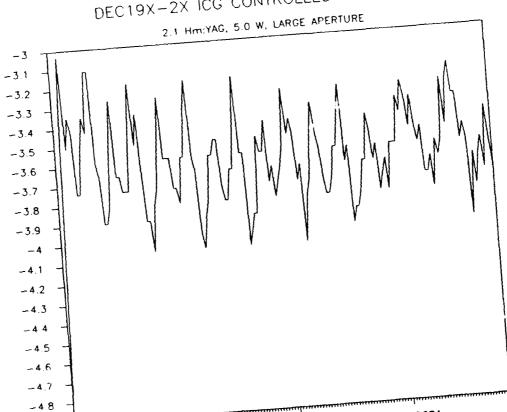
-7.450862

-18

-0.01465517

-2.463793

DEC19X-2X ICG CONTROLLED AT 70, 10s



-1.976293

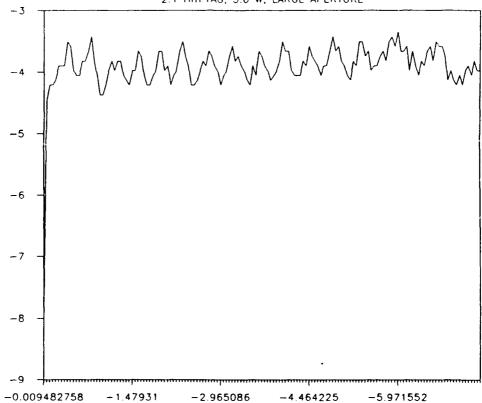
-4.9

-0 01465517

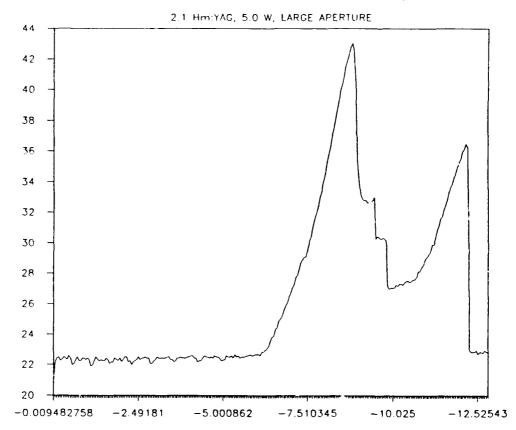
-3.962069

-5.96681

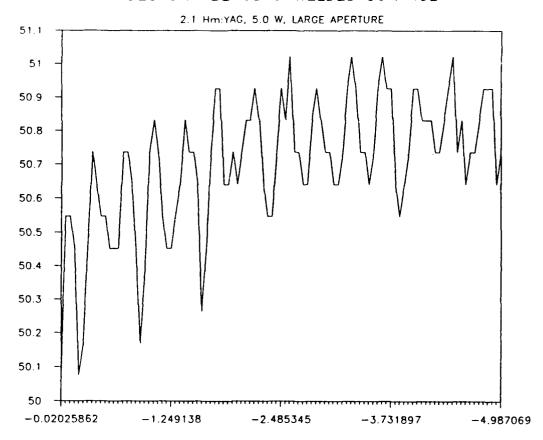
DEC19Y- 2X ICG CONTROLLED AT 80, 10s



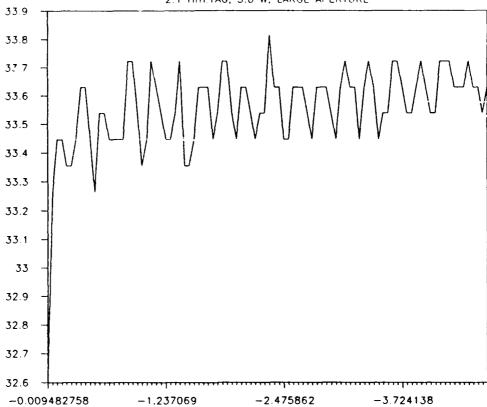
DEC19Z-2X ICG CONTROLLED AT 100, 10s



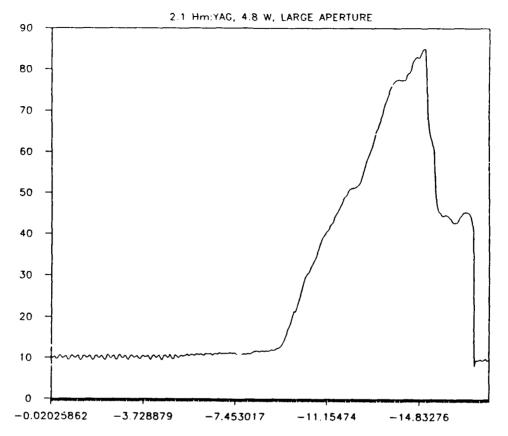
# DEC19AA-BLOOD UNWELDED CONTROL



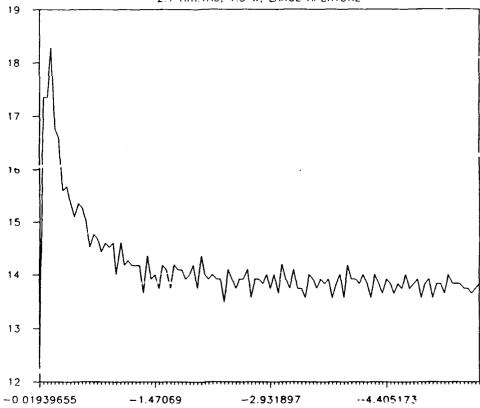
# DEC19AB-BLOOD CONTROLLED AT 50, 10s



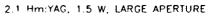
# DEC21A-ONE STRIP UNWELDED CONTROL

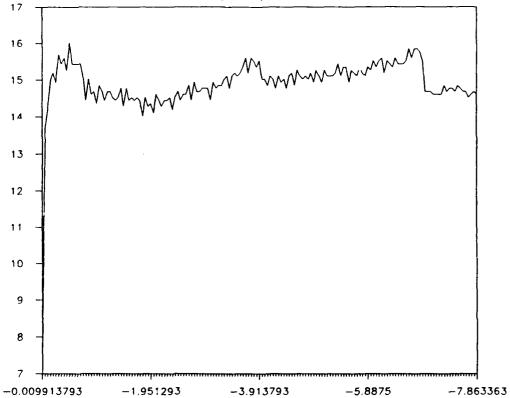


# DEC21AA1-NO CHROMO CONTROLLED AT 70,10s

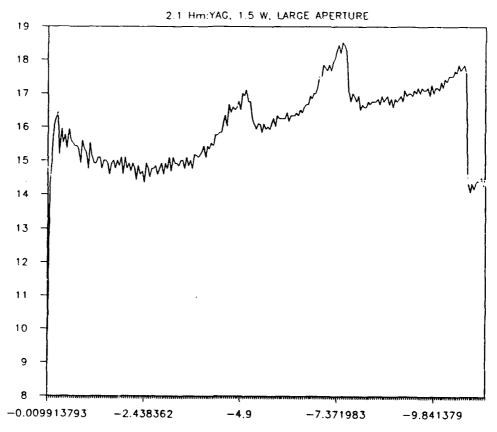


### DEC21AB-NO CHROMO CONTROLLED AT 80, 10s

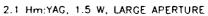


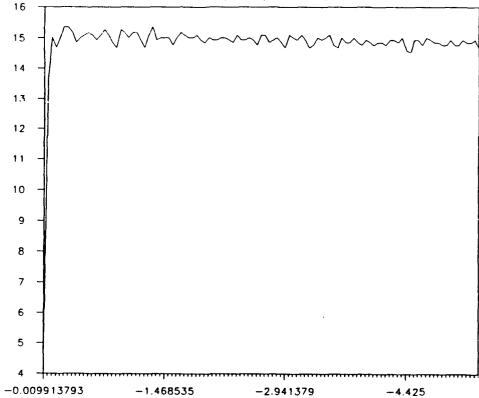


# DEC21AC-NO CHROMO CONTROLLED AT 100,10s

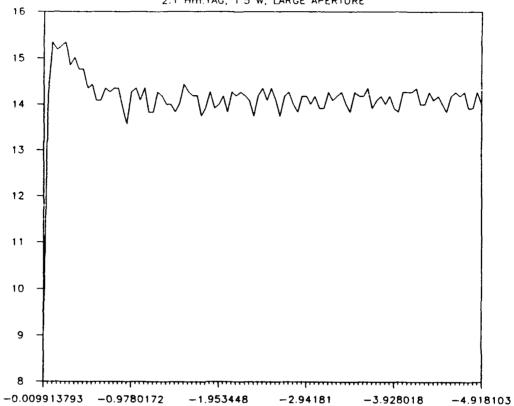


### DEC21AD-INDIA INK UNWELDED CONTROL



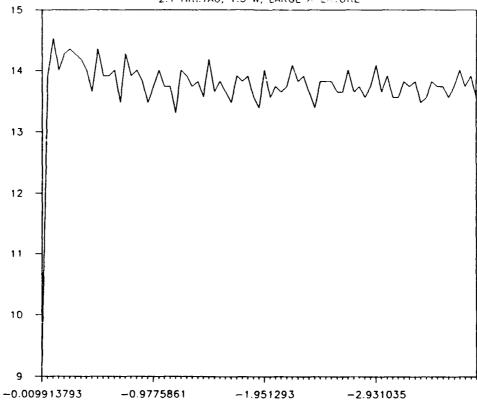


### DEC21AE-INDIA INK CONTROLLED AT 50,10s

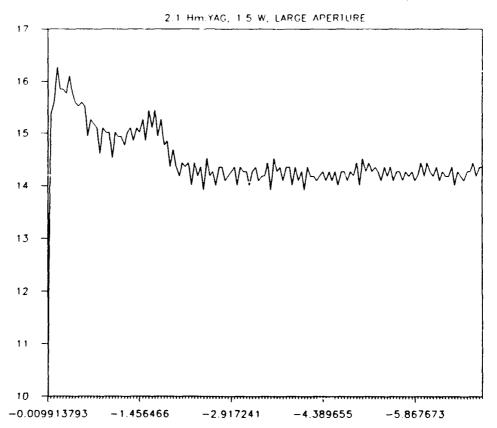


#### DEC21AF-INDIA INK CONTROLLED AT 60, 10s

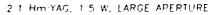


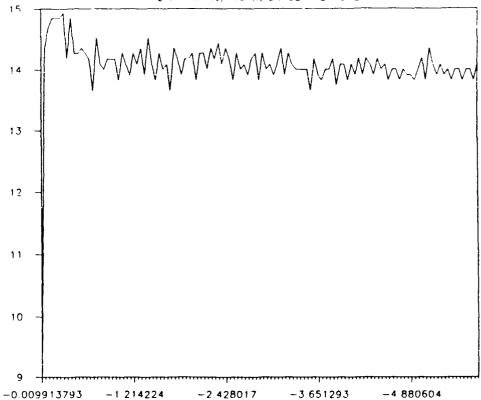


#### DEC21AG-INDIA INK CONTROLLED AT 70, 10s

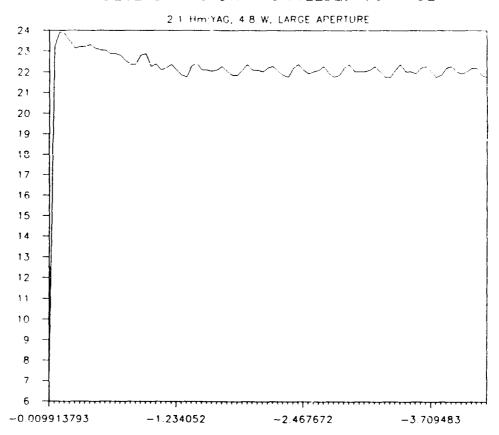


### DEC21AH-INDIA INK CONTROLLED AT 80, 10s

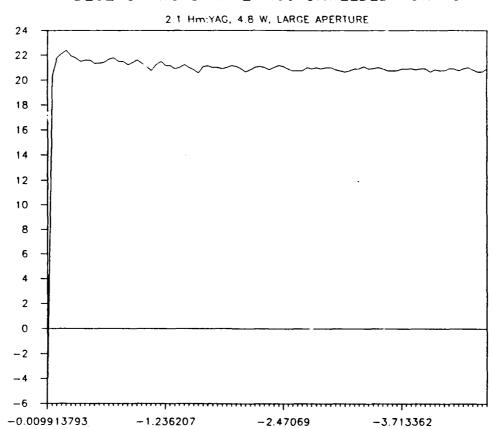




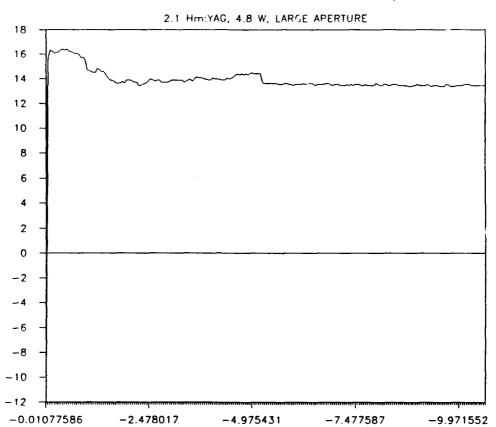
#### DEC21B-TWO STRIP UNWELDED CONTROL



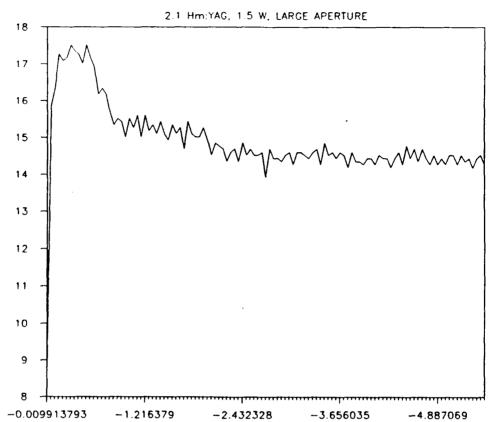
### DEC21C-TWO STRIP 2X ICG UNWELDED CONTRO



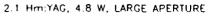
DEC21D-2X ICG CONTROLLED AT 50, 10s

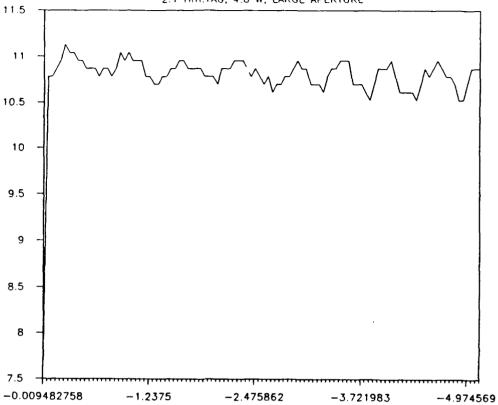


## DEC21AI-INDIA INK CONTROLLED AT 100,10s

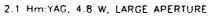


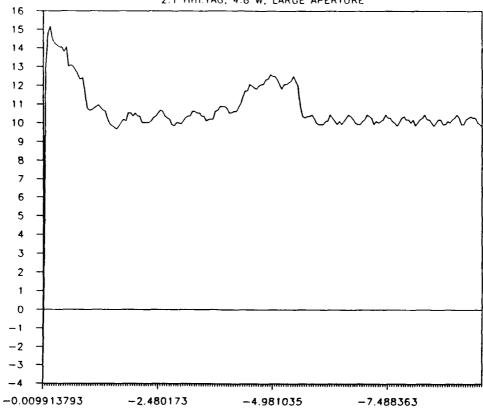
# DEC21E-2X ICG CONTROLLED AT 60, 10s



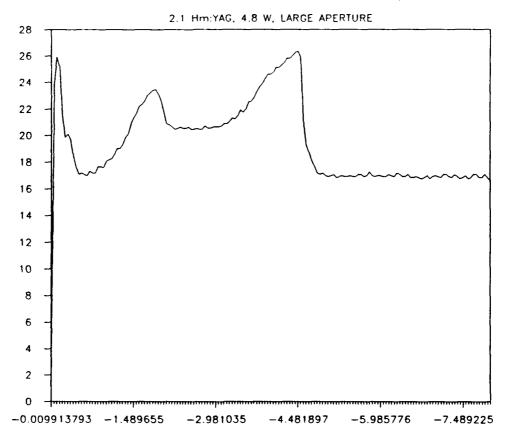


### DEC21F-2X ICG CONTROLLED AT 70, 10s

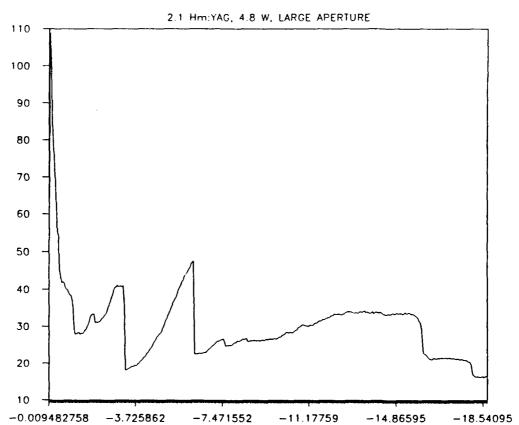




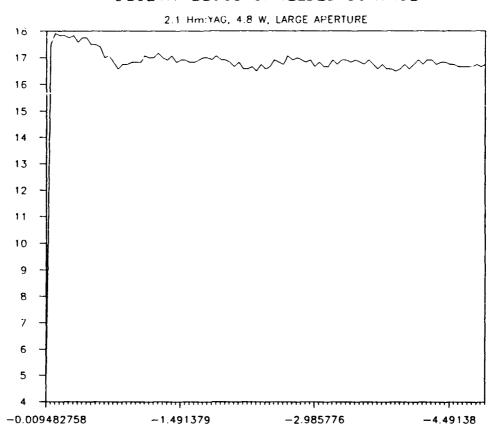
#### DEC21G-2X ICG CONTROLLED AT 80, 10s



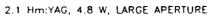
#### DEC21H-2X ICG CONTROLLED AT 100, 10s

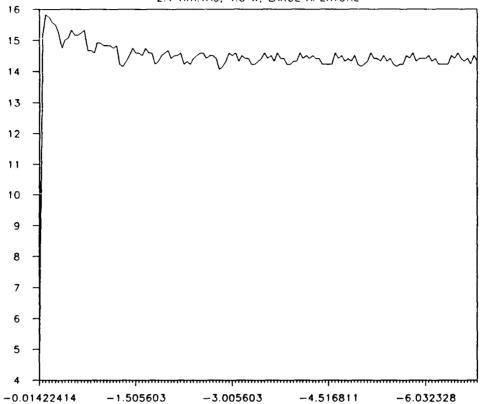


### DEC21H-BLOOD UNWELDED CONTROL

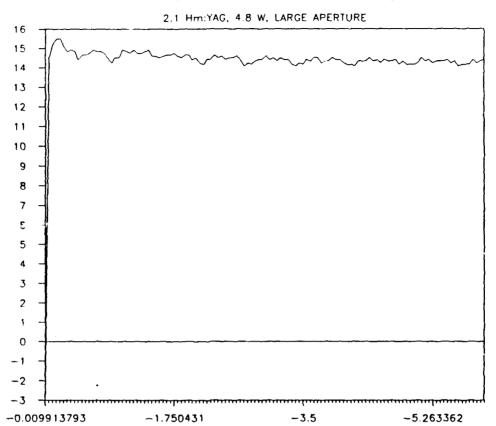


# DEC21J-BLOOD CONTROLLED AT 50, 10s



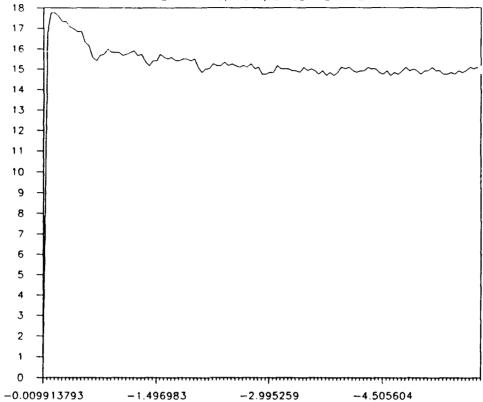


# DEC21K-BLOOD CONTROLLED AT 60, 10s

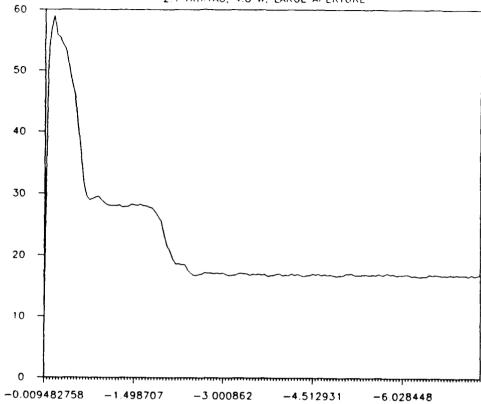


DEC21L- BLOOD CONTROLLED AT 70, 10s



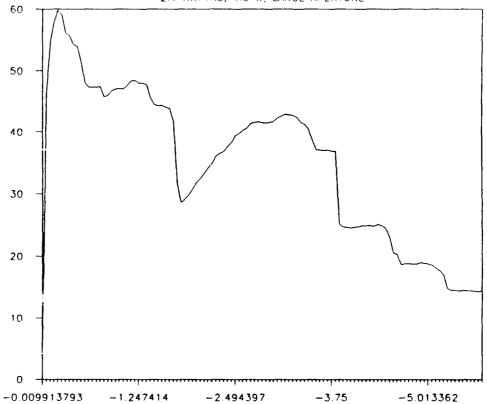


DEC21M- BLOOD CONTROLLED AT 80, 10s



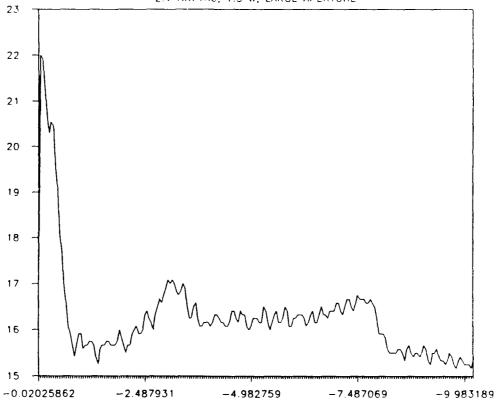
# DEC21N-BLOOD CONTROLLED AT 100, 10s





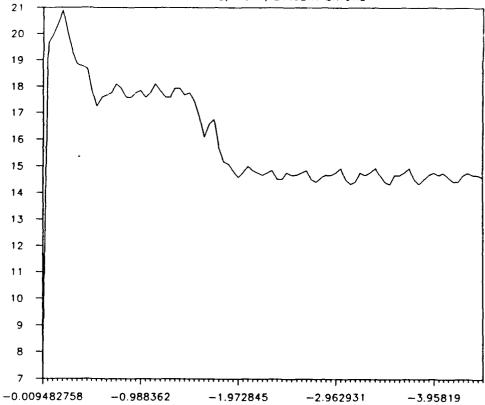
#### DEC2101-BLOOD CONTROLLED AT 50, 10s





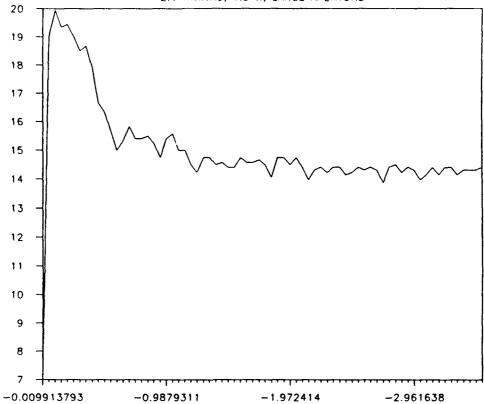
# DEC21P-BLOOD CONTROLLED AT 60, 10s

2.1 Hm:YAG, 1.5 W, LARGE APERTURE

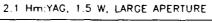


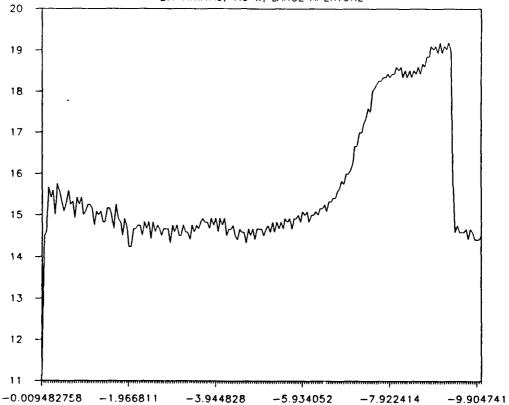
#### DEC21Q-BLOOD CONTROLLED AT 70, 10s

2.1 Hm:YAG, 1.5 W, LARGE APERTURE

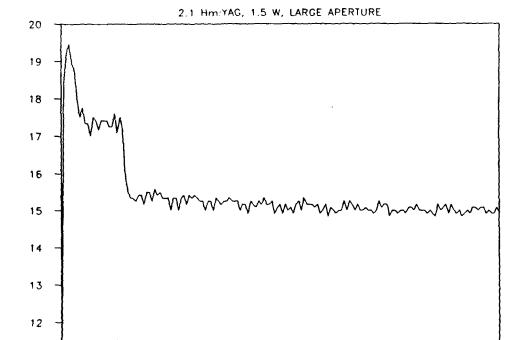


#### DEC21R-BLOOD CONTROLLED AT 80, 10s





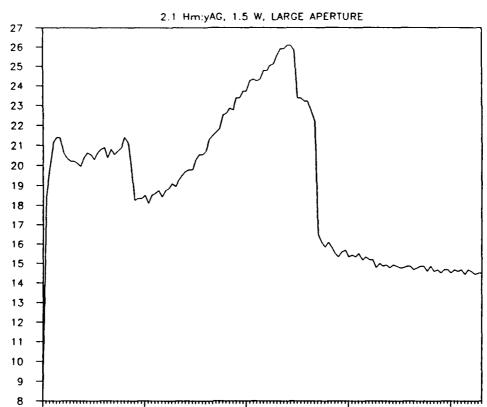
# DEC21T-2X ICG CONTROLLED AT 50, 10s



-0.009482758 -1.462069 -2.927155 -4.405604 -5.888362 +7.371121

11

# DEC21S-BLOOD CONTROLLED AT 100, 10s



-2.938362

-4.421121

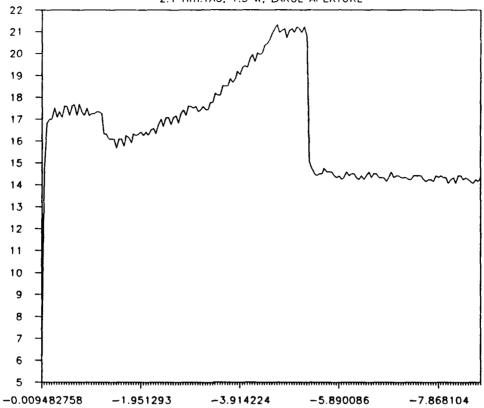
-5.910776

-0.009913793

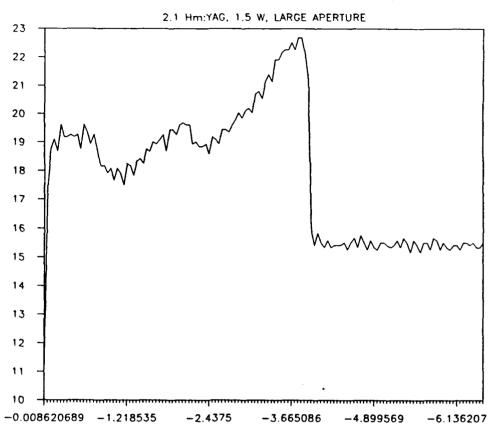
-1.467673

#### DEC21U-2X ICG CONTROLLED AT 60, 10s

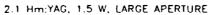
2.1 Hm:YAG, 1.5 W, LARGE APERTURE

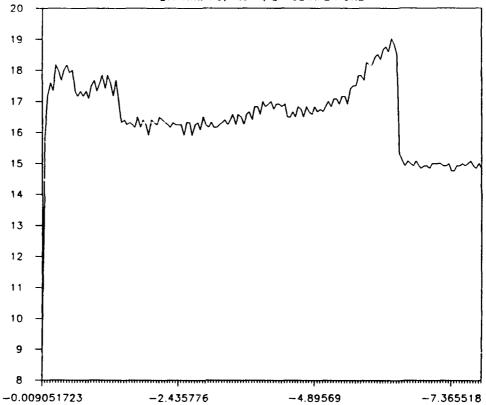


# DEC21V-2X ICG CONTROLLED AT 70, 10s

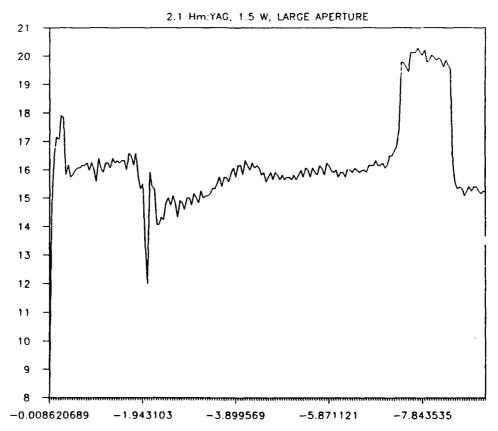


# DEC21W-2X ICG CONTROLLED AT 80, 10s



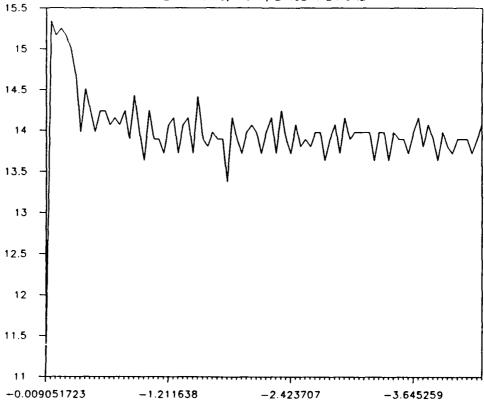


#### DEC21X-2X ICG CONTROLLED AT 100, 10s

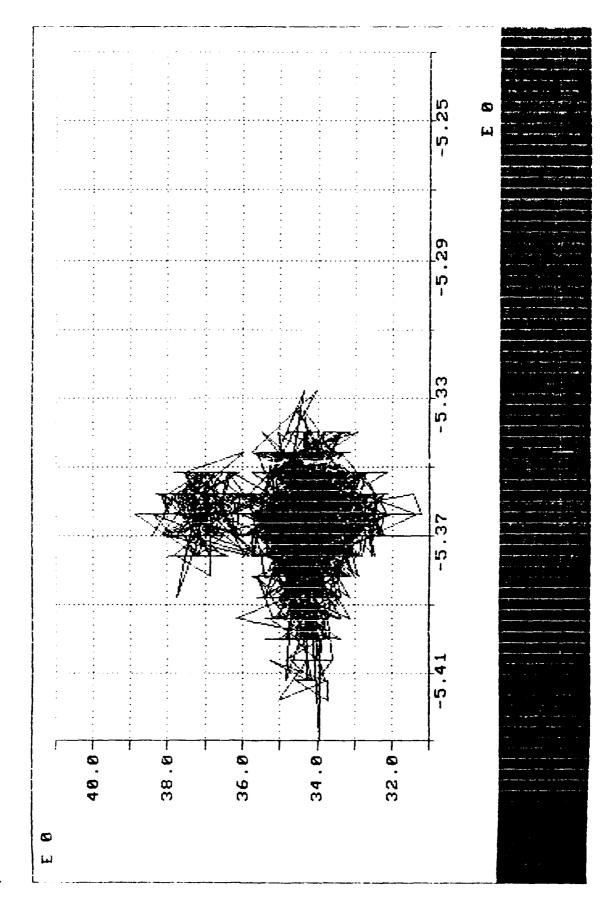


#### DEC21Y-NO CHROMO CONTROLLED AT 50, 10s



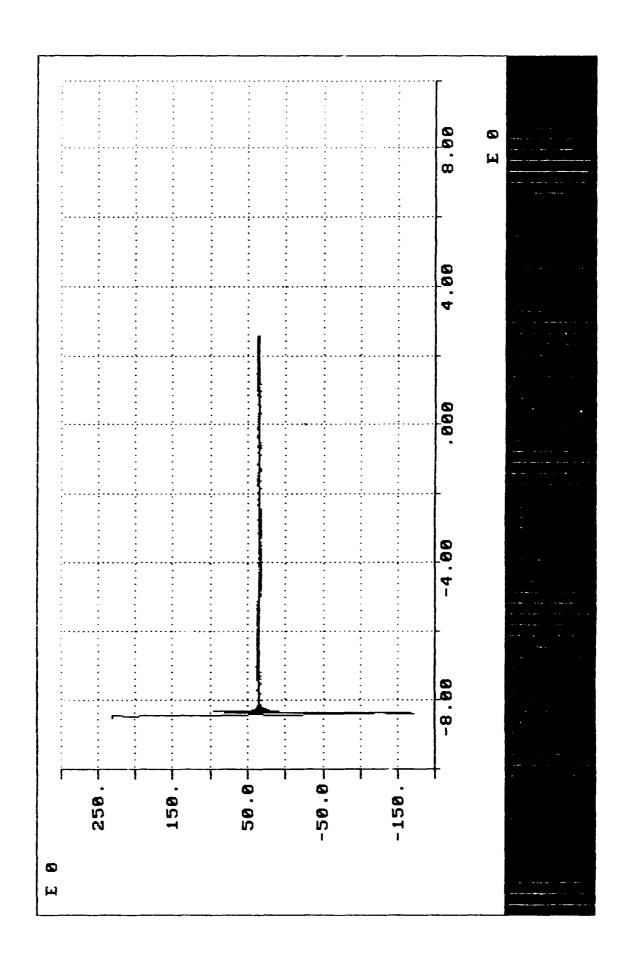


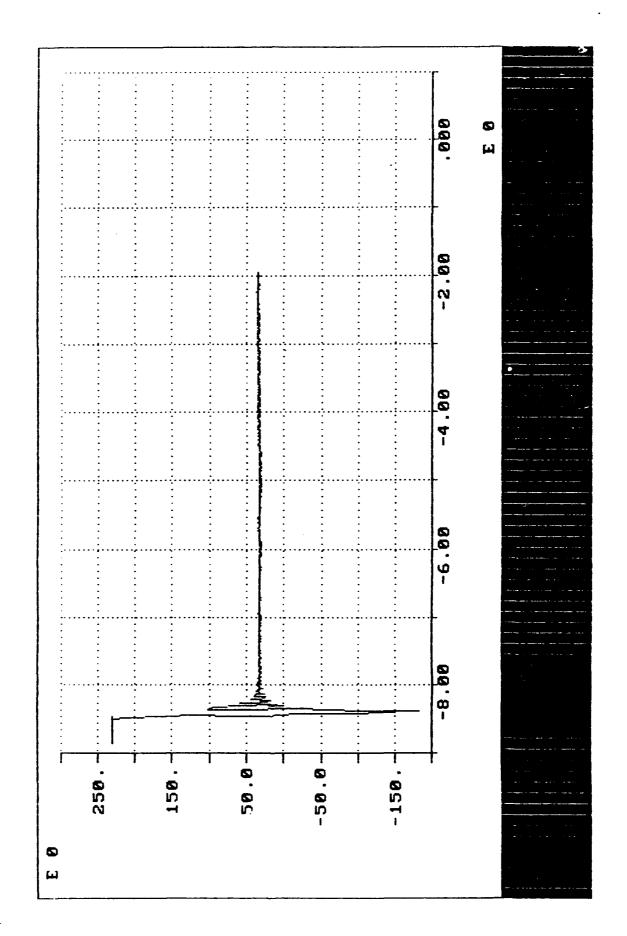
# **Appendix B- Excluded Data**

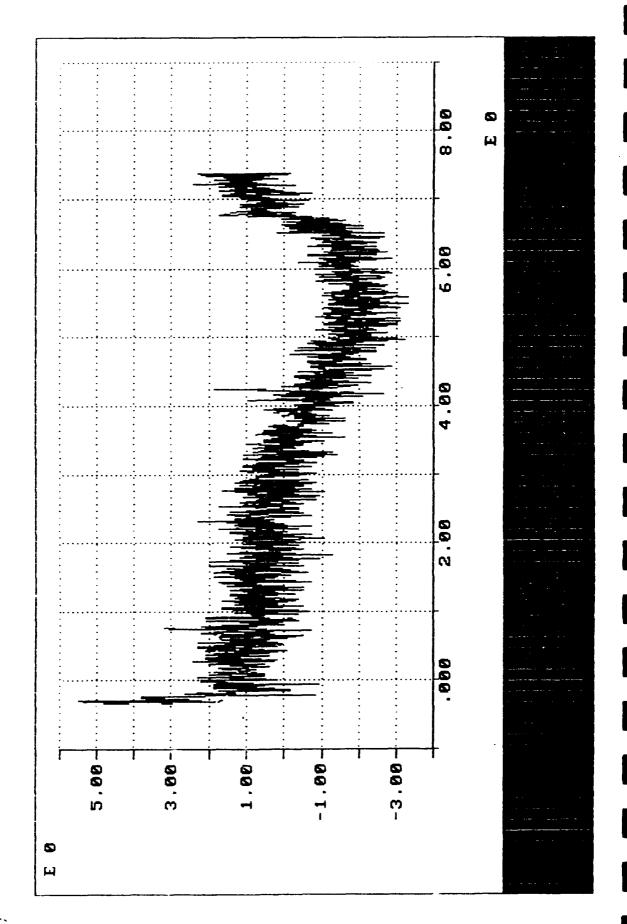


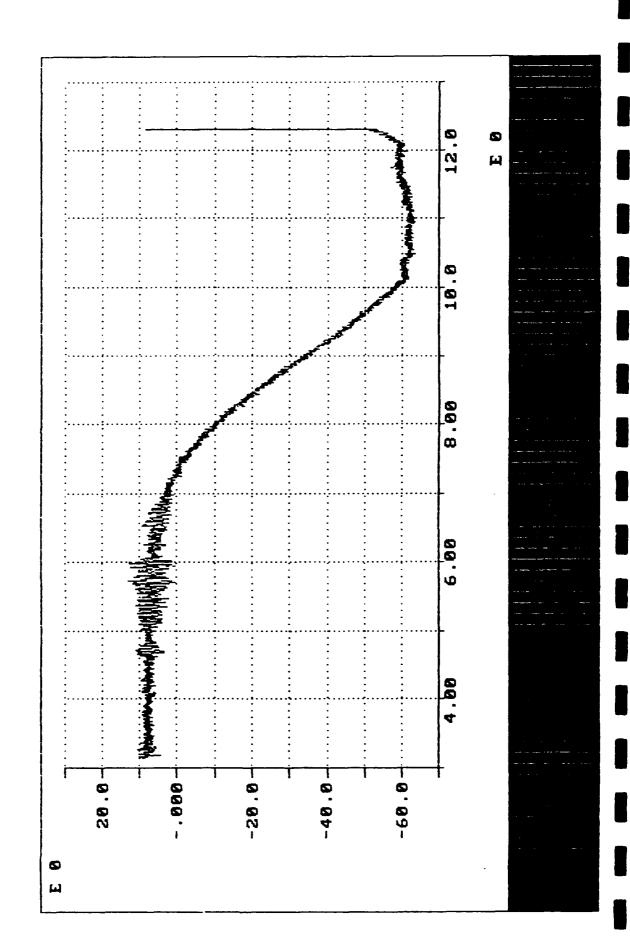
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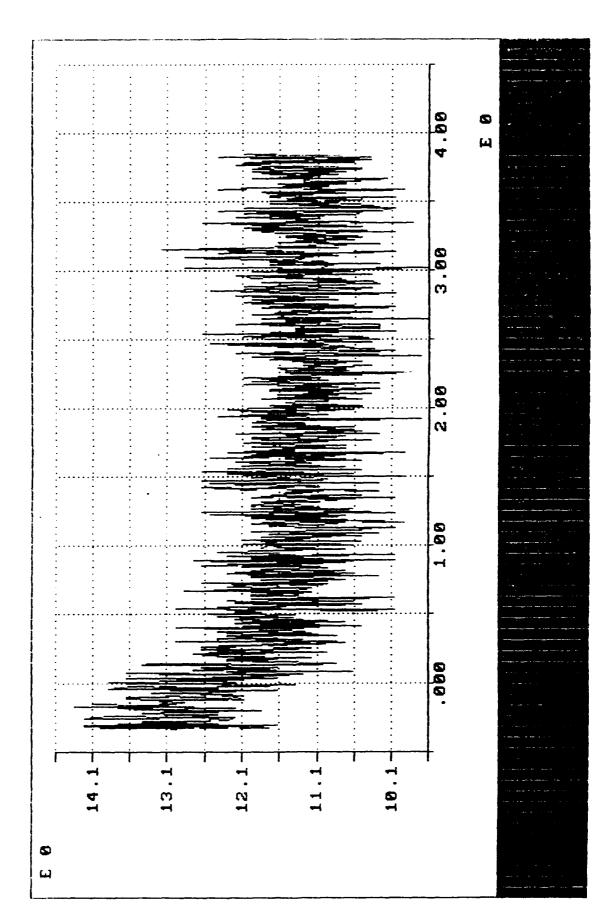


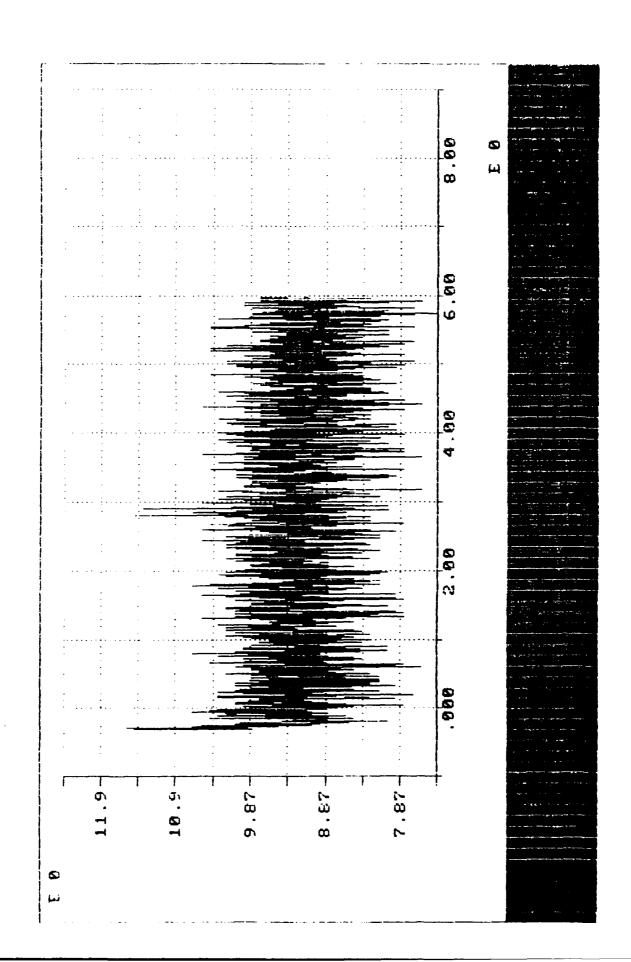


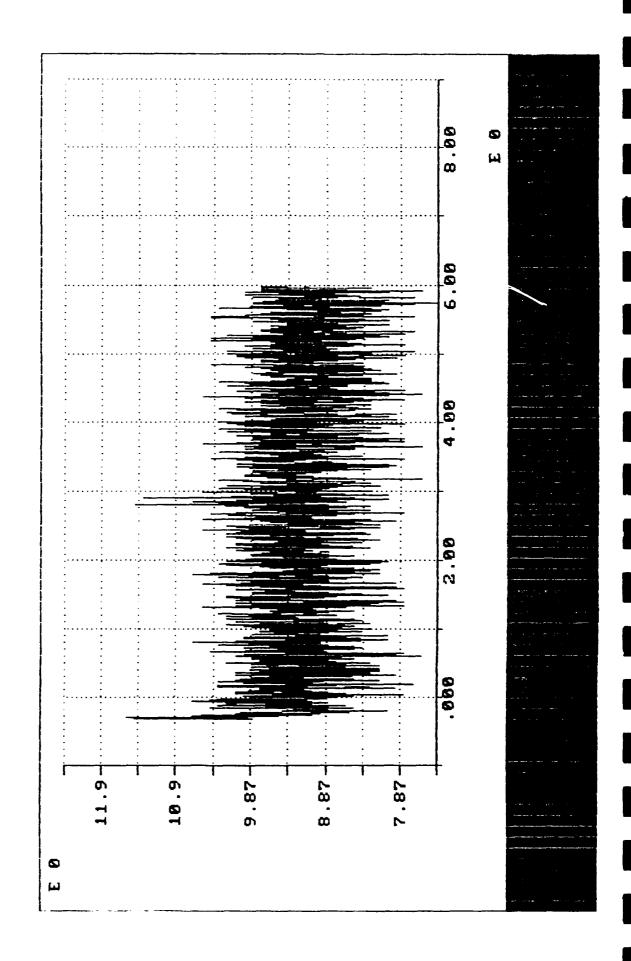




DIE 100

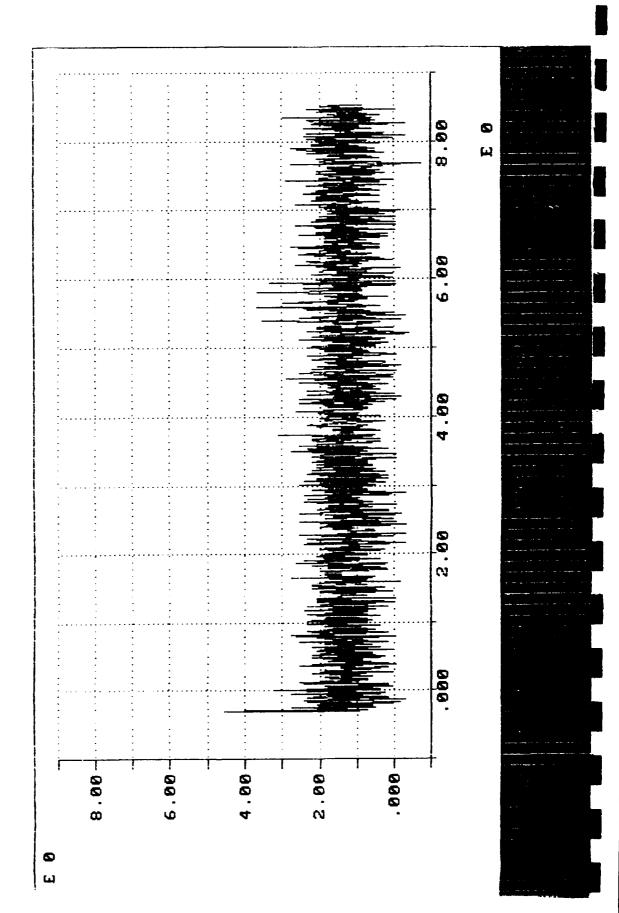




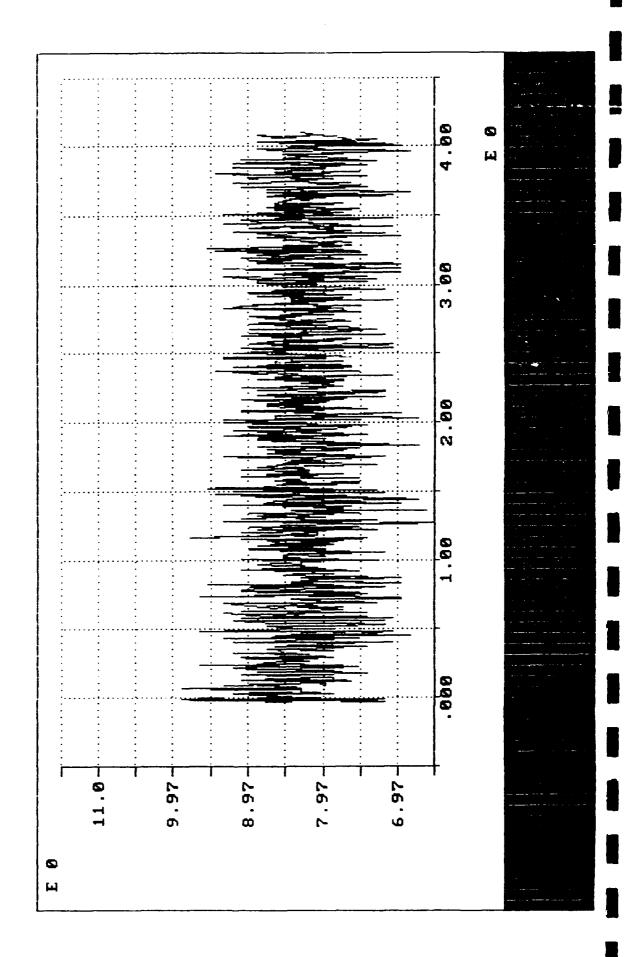


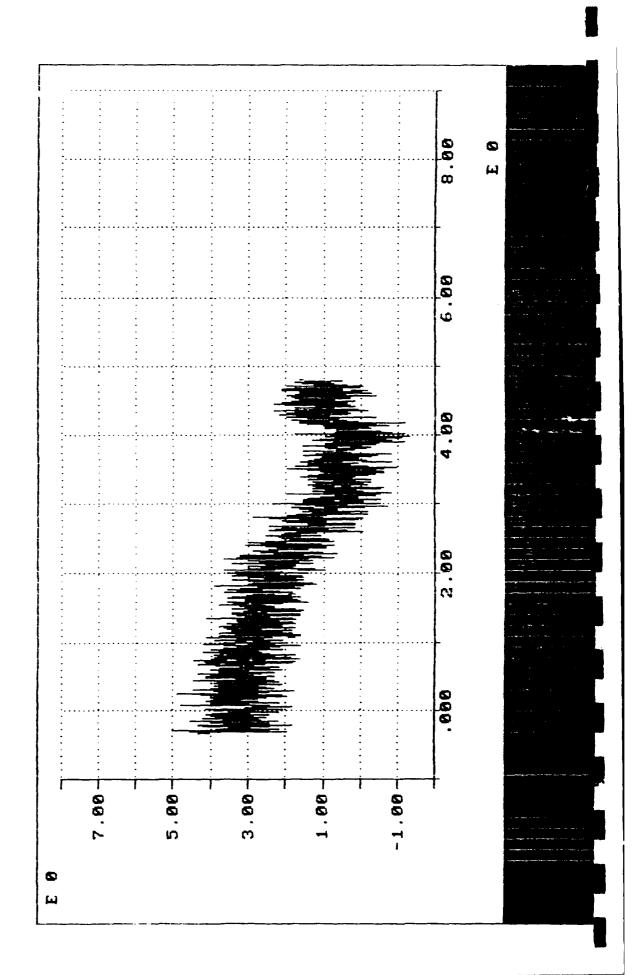
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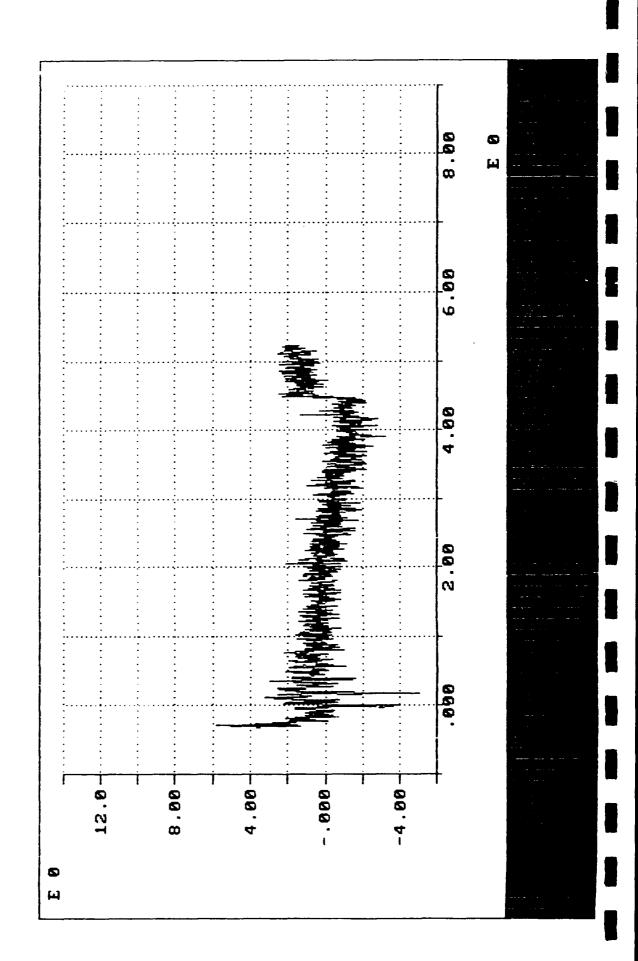


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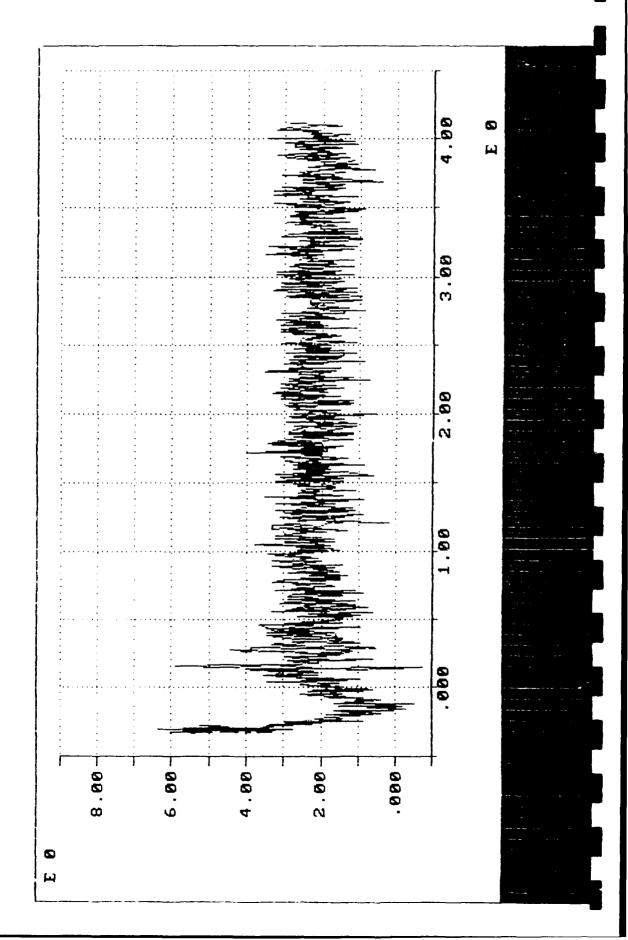




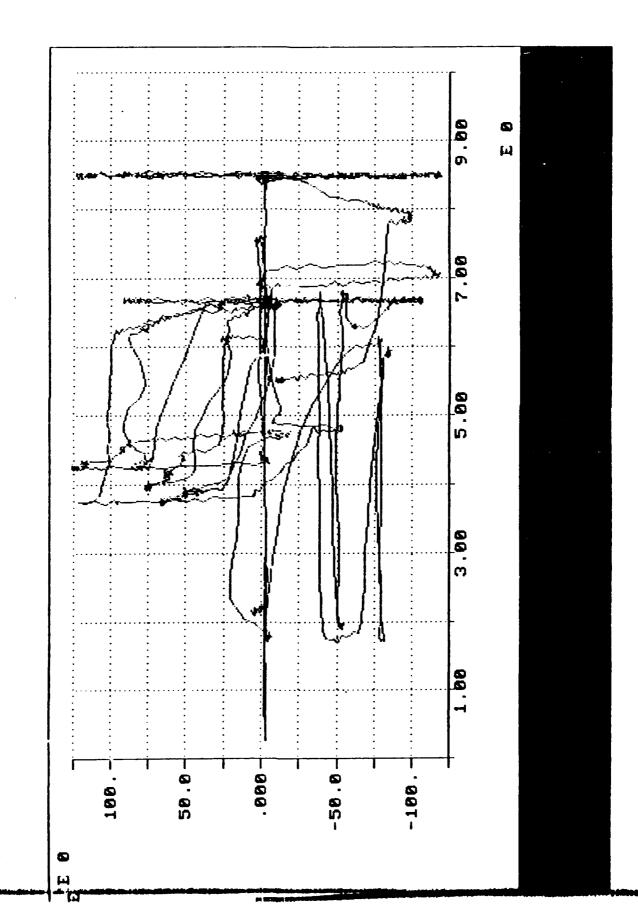
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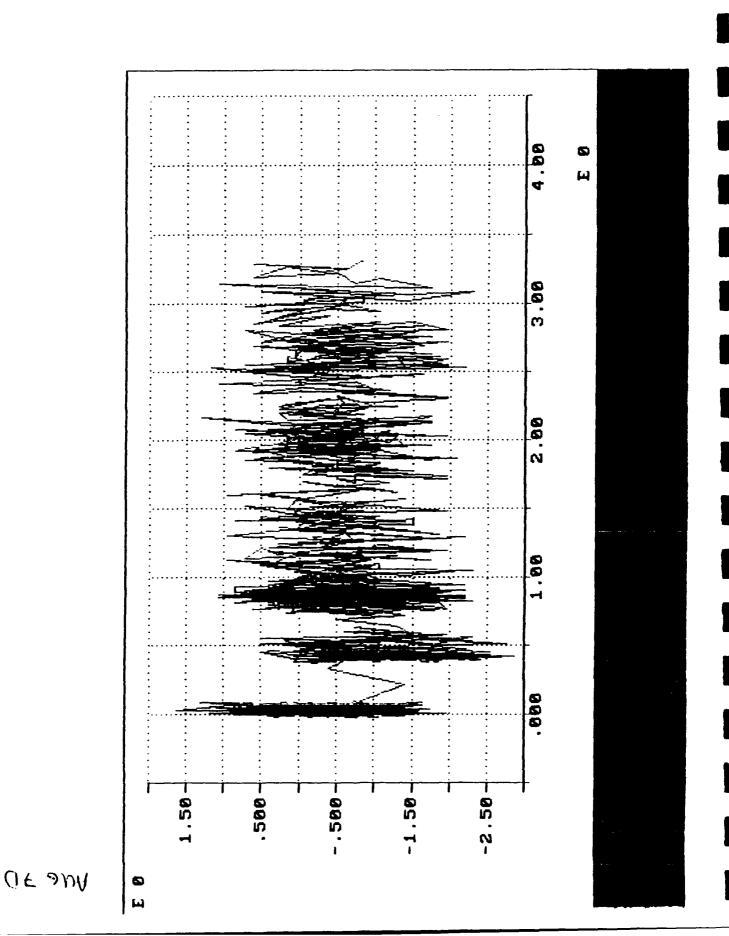
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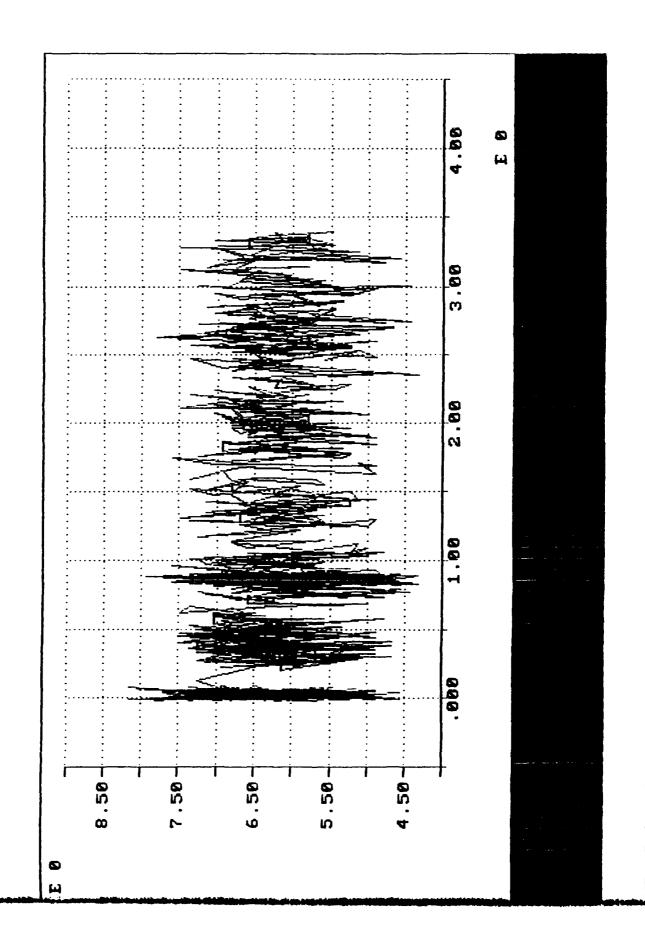


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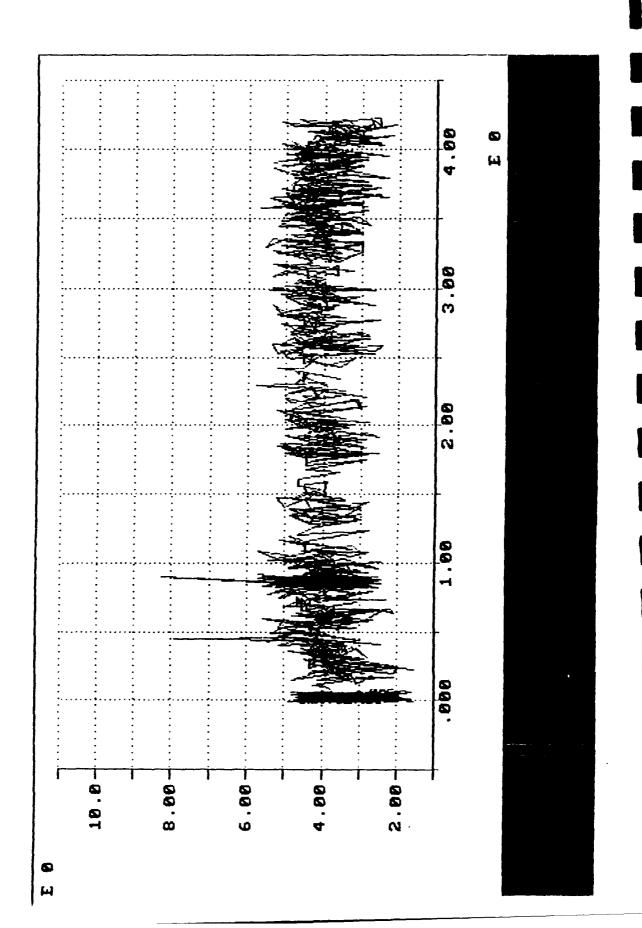


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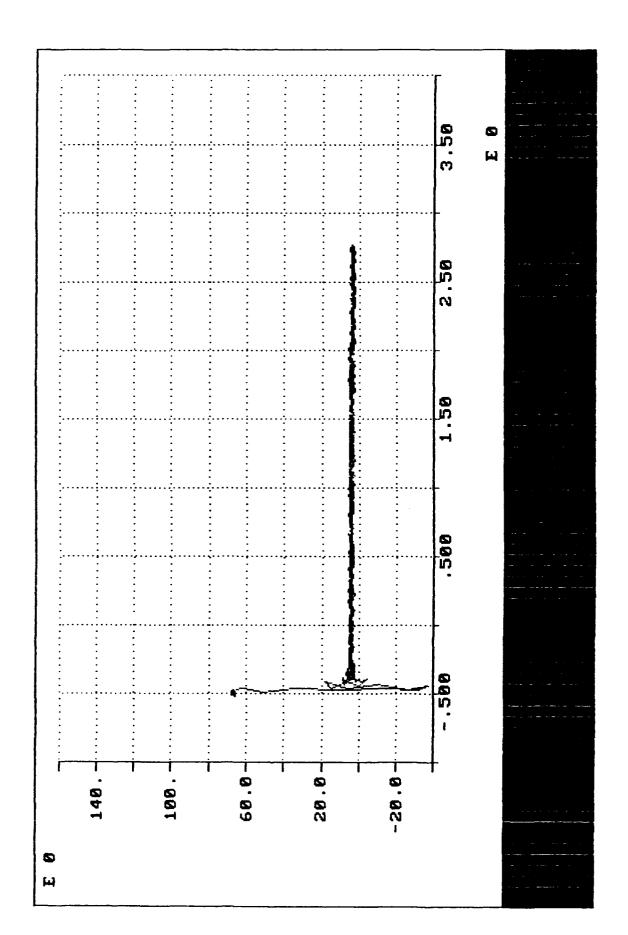


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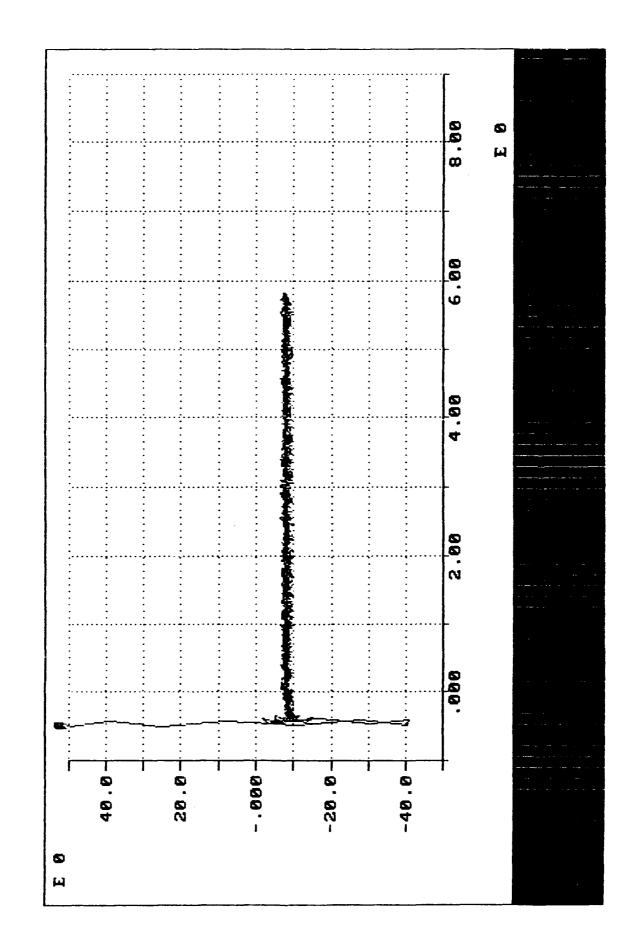


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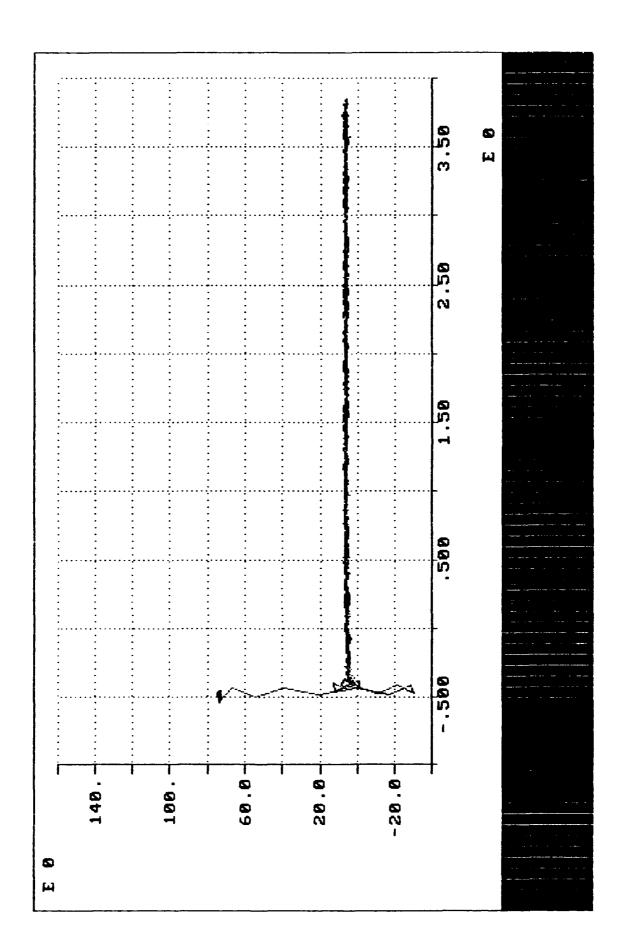
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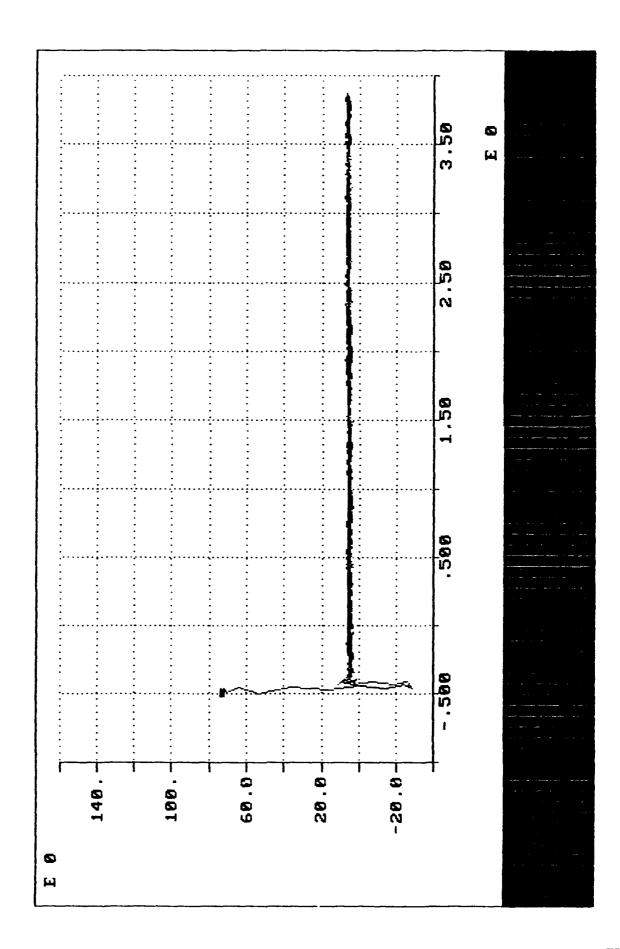
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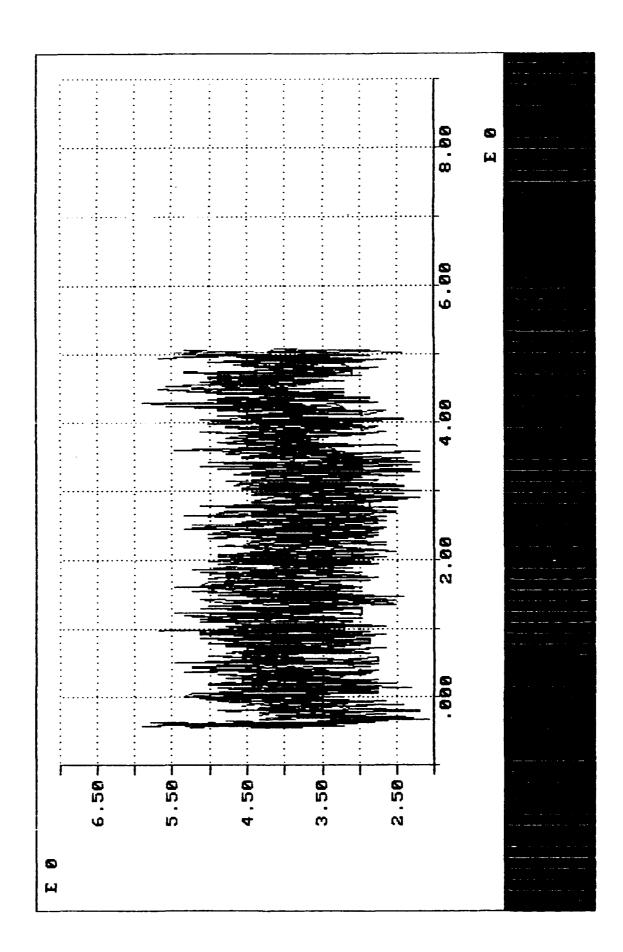
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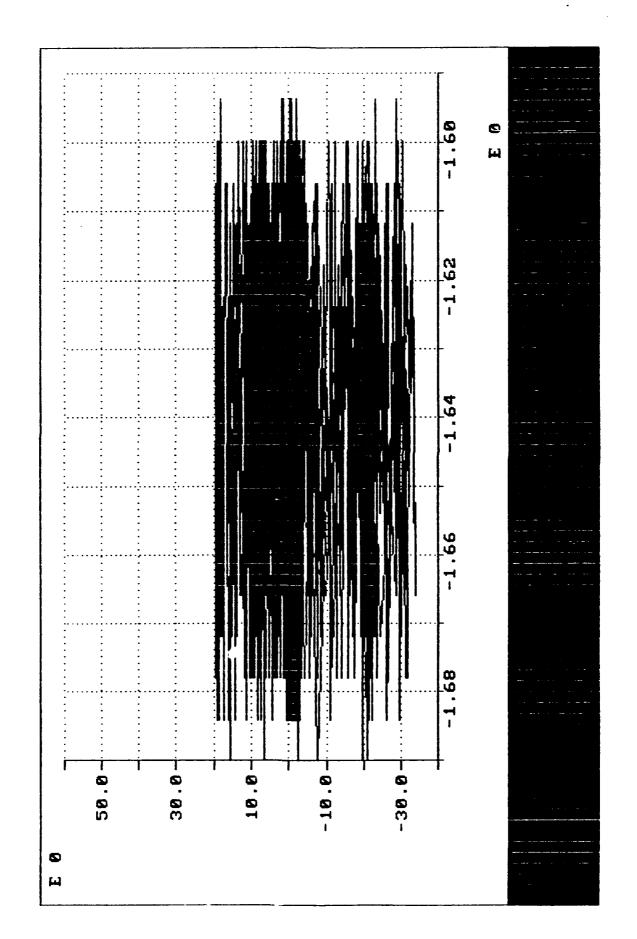


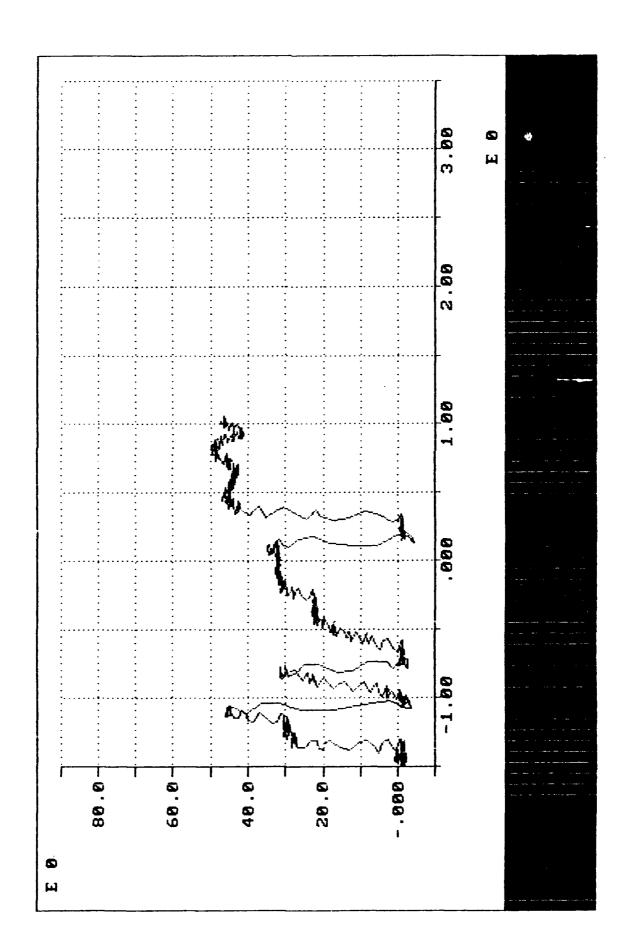
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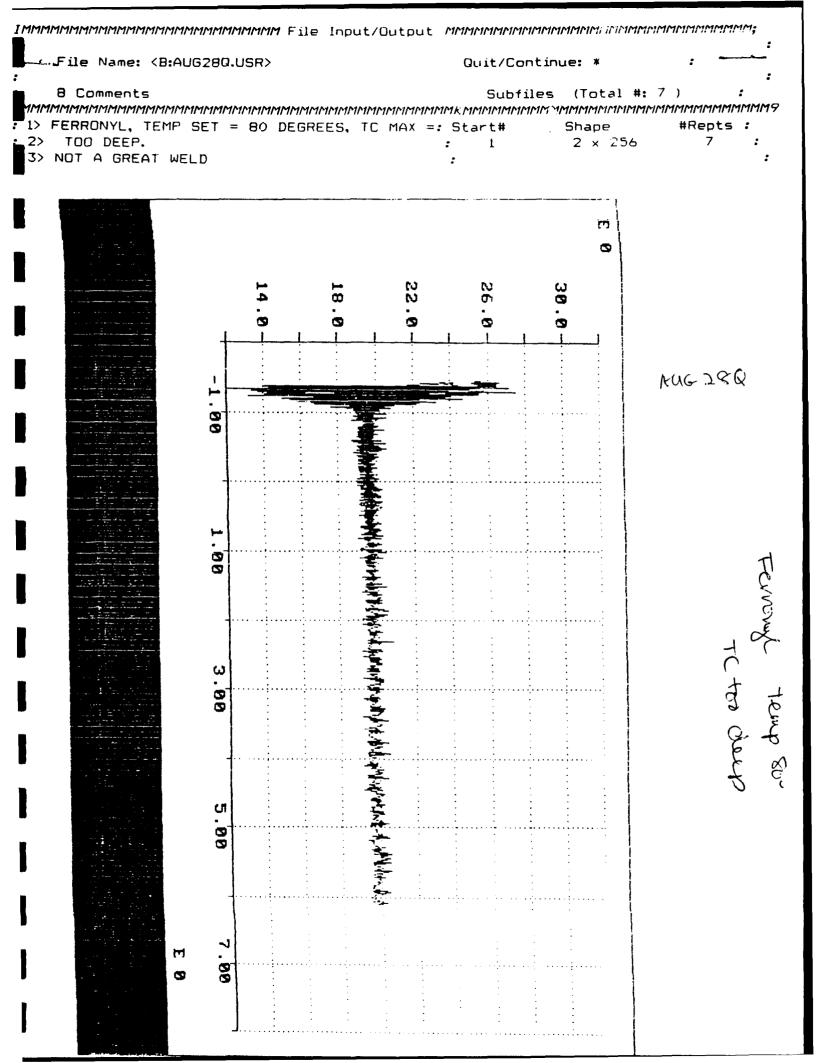
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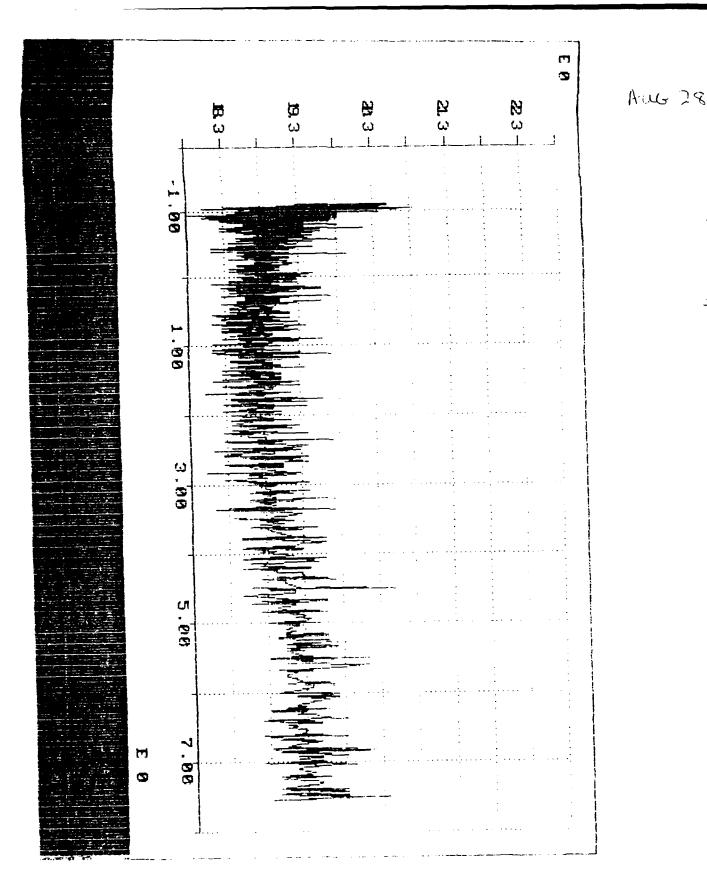




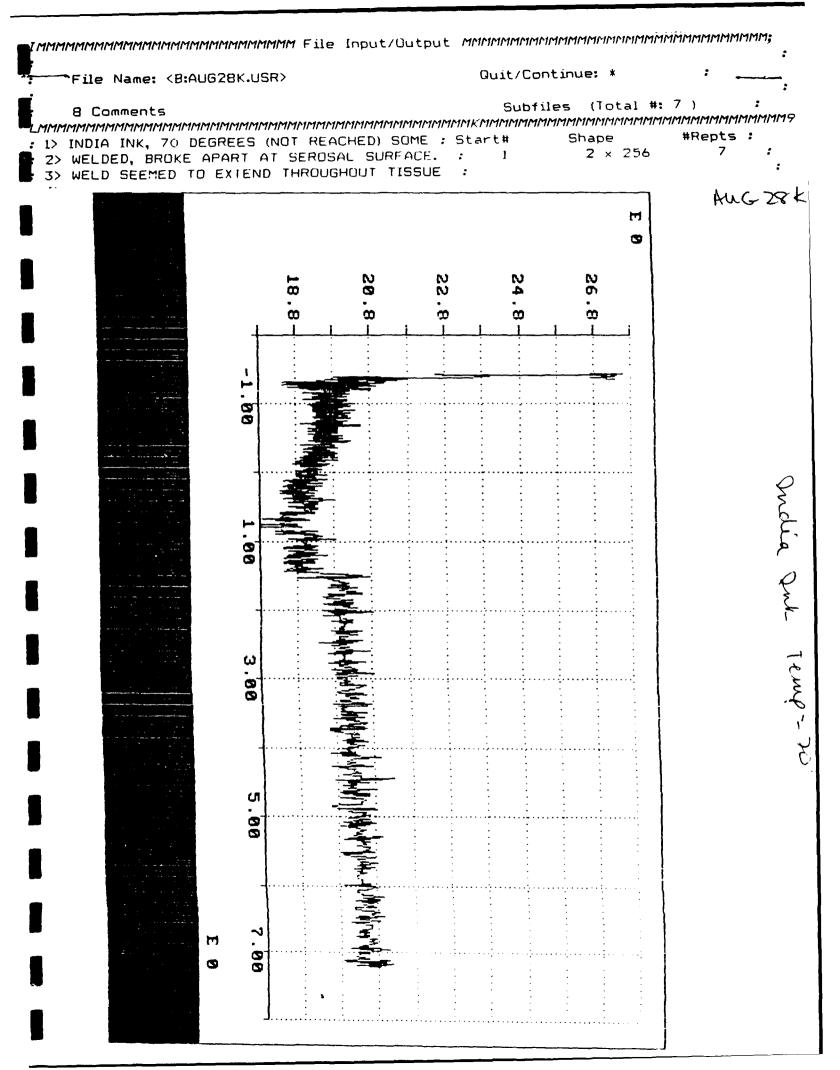
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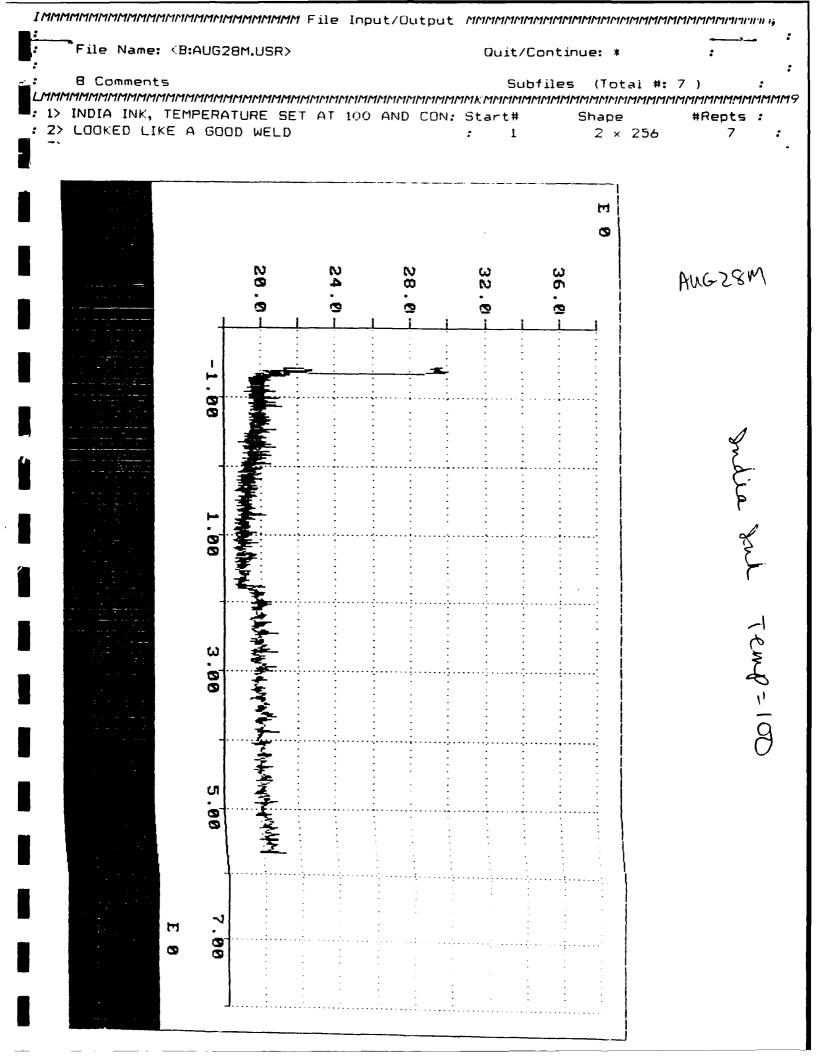
File Name: <B:AUG28AA.USR> Quit/Continue: \* 8 Comments Subfiles (Total #: 5 ) 1> LARGE APERTURE, INDIA INK, 50 DEGREES : Start# Shape #Repts : 2> WELDED 2 × 256 AUG28AA N

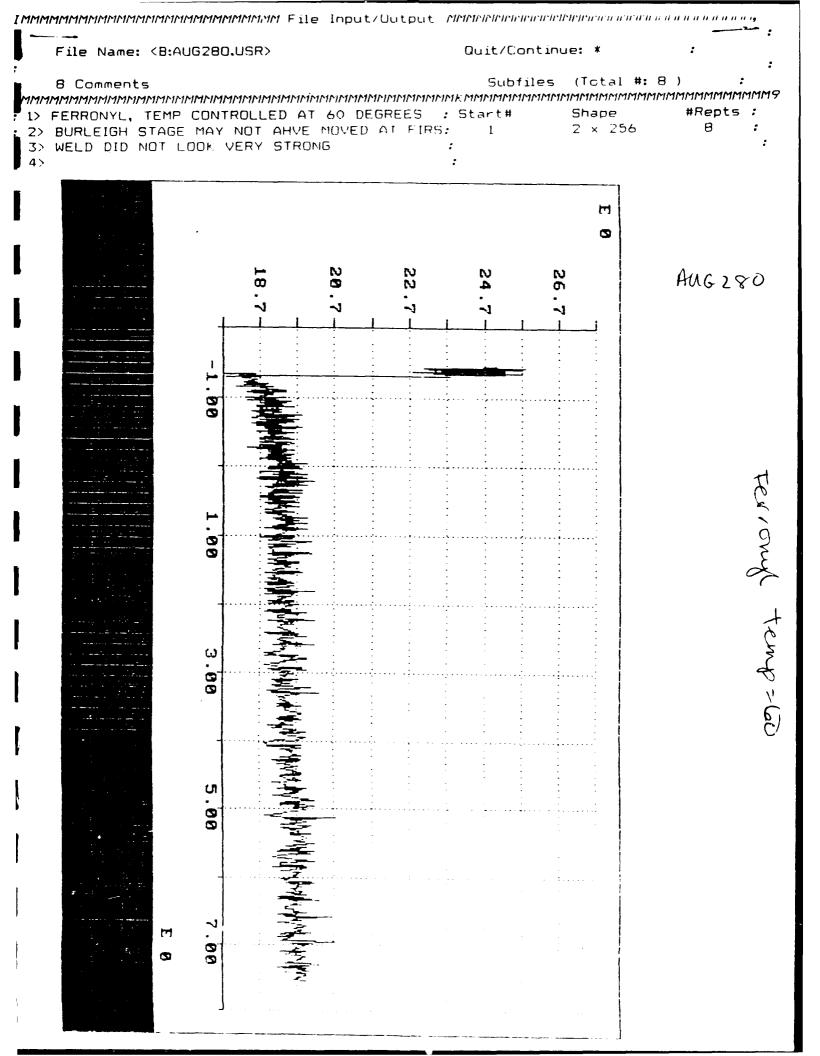




File Name: <8:AUG26U.JSR> Quit/Continue: \* 8 Comments Subfiles (Total #: 7 ) : 1> FERRONYL, TEMP SET AND CONTROLLED AT 80 D: Start# Shape #Repts : 3> LOOKED STRONGER THAN THE 70 DEGREE AND ST:  $2 \times 256$ 7 WELDS FROM THIS MORNING. H AUG 28 4 3 12

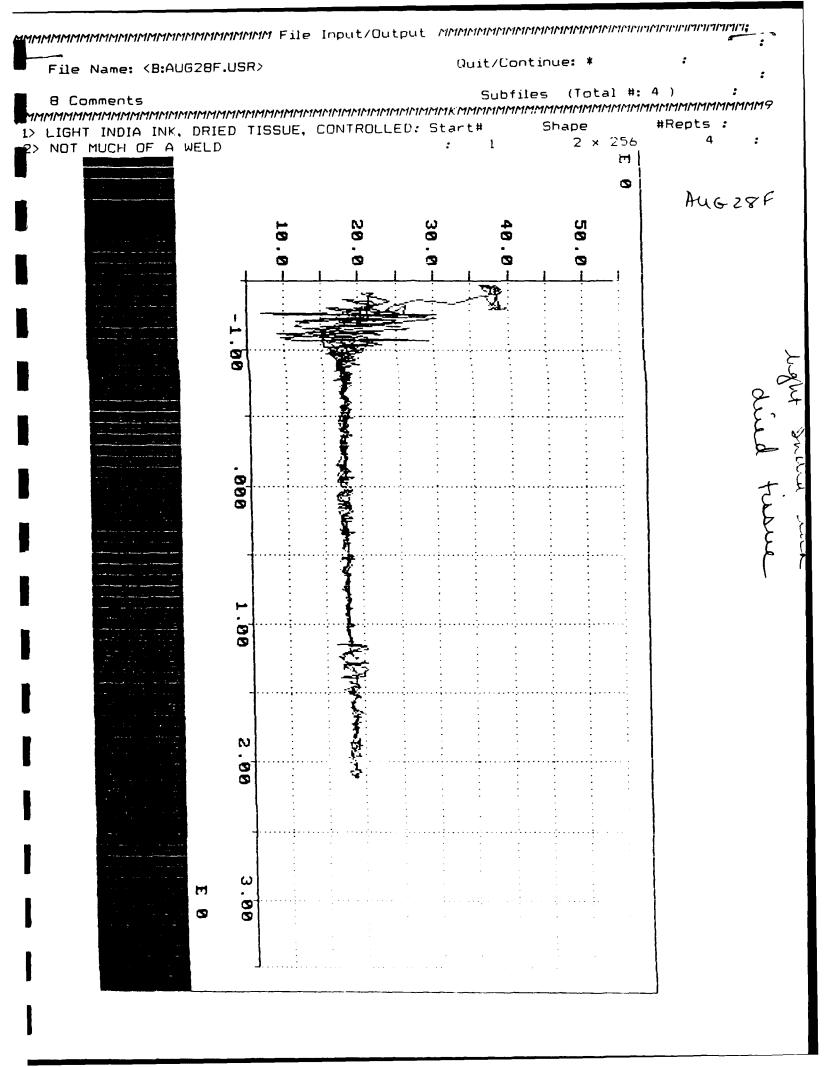






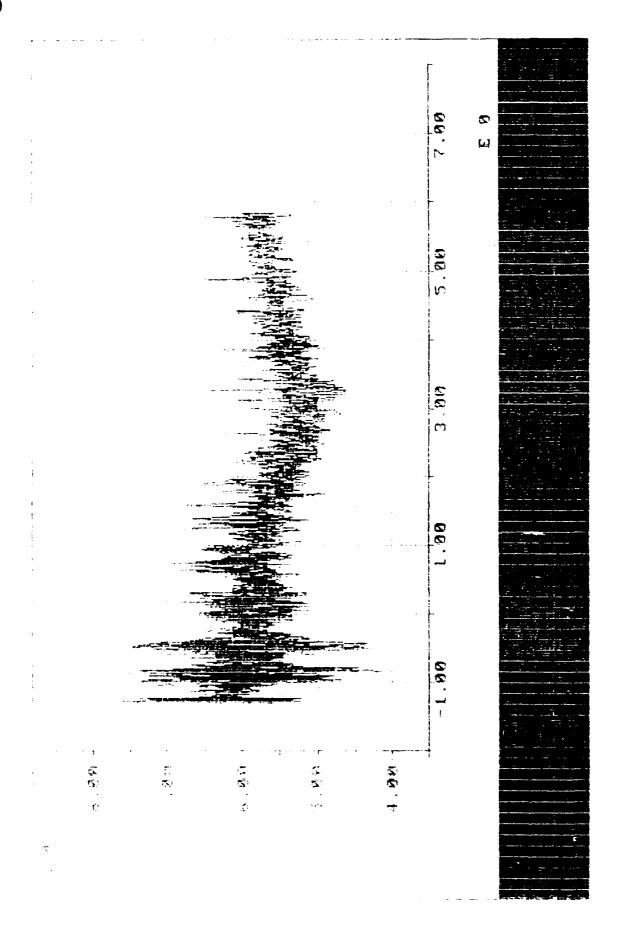
-File Name: <B:AUG28P.USR> Quit/Continue: \* 8 Comments Subfiles (Total #: 8 ) 1> FERRONYL 70 DEGREES LITTLE DESSICATION AN: Start# Shape #Repts : 2> A WELD, STRONGER THAN 60 DEGRES, BUT NOT :  $2 \times 256$ 3> FERRONYL BARELY VISIBLE 3 1emp=700

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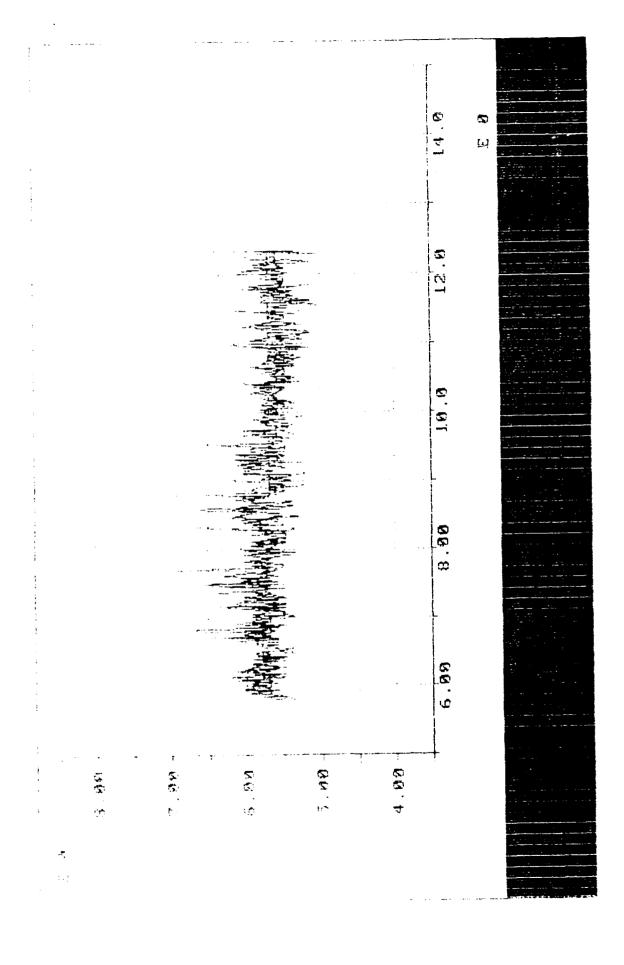
Quit/Continue: \* - File Name: <B:AUG2BJ.USR> Subfiles (Total #: 6 ) 1> INDIA INK, TEMP = 60 DEGREES, SLIGHT DESS: Start# Shape 6 2> LOOKS LIKE A WELD, TORE AT SEROSAL SURFCE:  $2 \times 256$ Auc 285 (3)

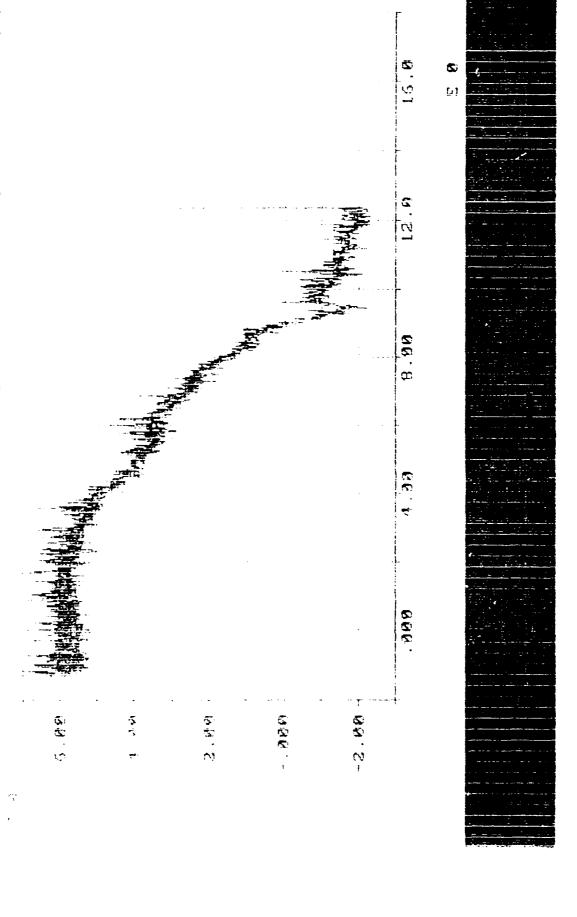
Quit/Continue: \* File Name: (B:AUG28D.USR) (Total #: 4 ) Subfiles #Repts : Shape : Start# INDIA INK LIGHT 2 x 256 1 NOT A GOOD WELD 3 Aug 200 India inte (light) . 500 500 5

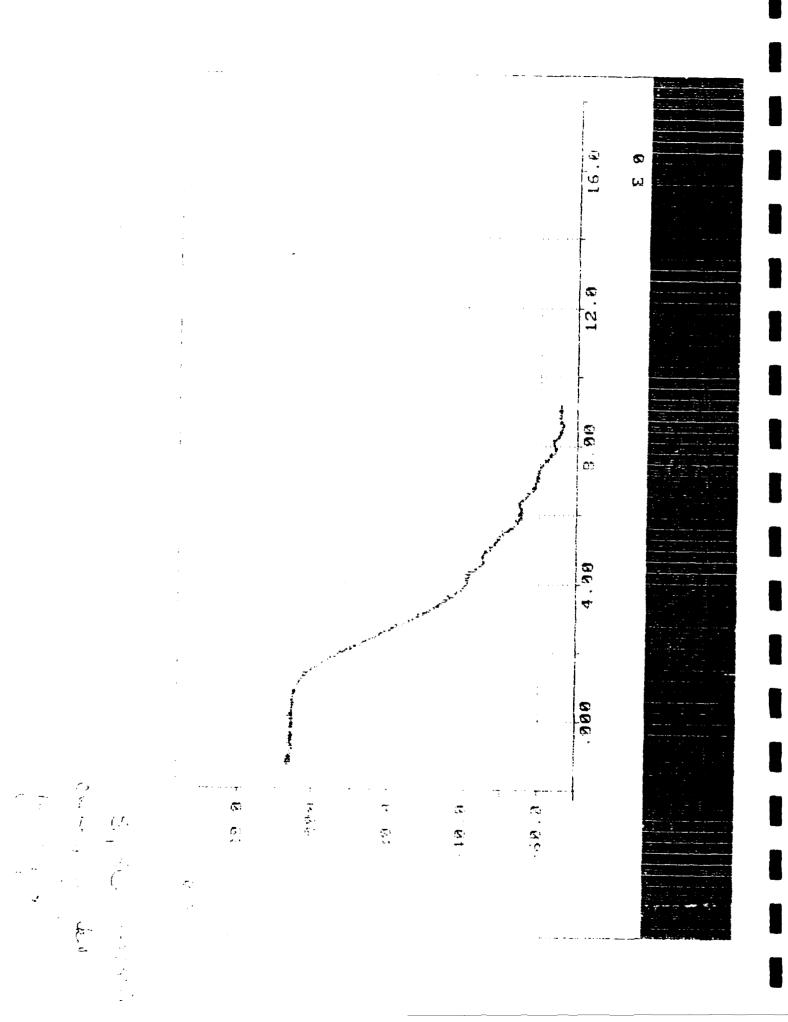


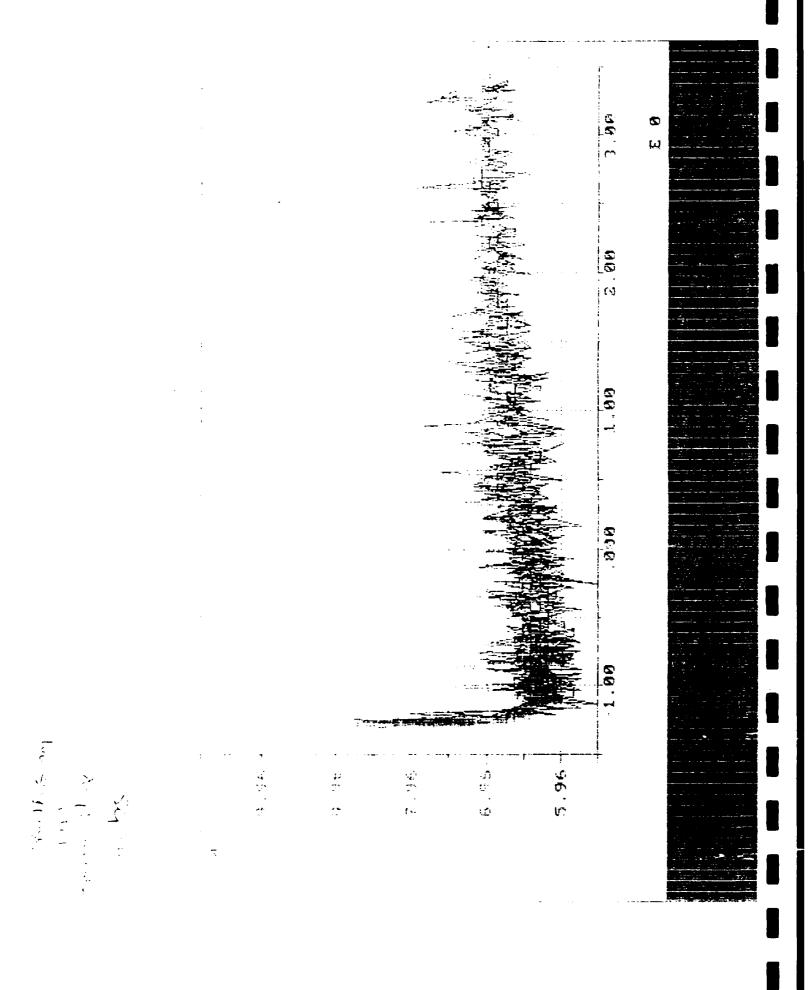
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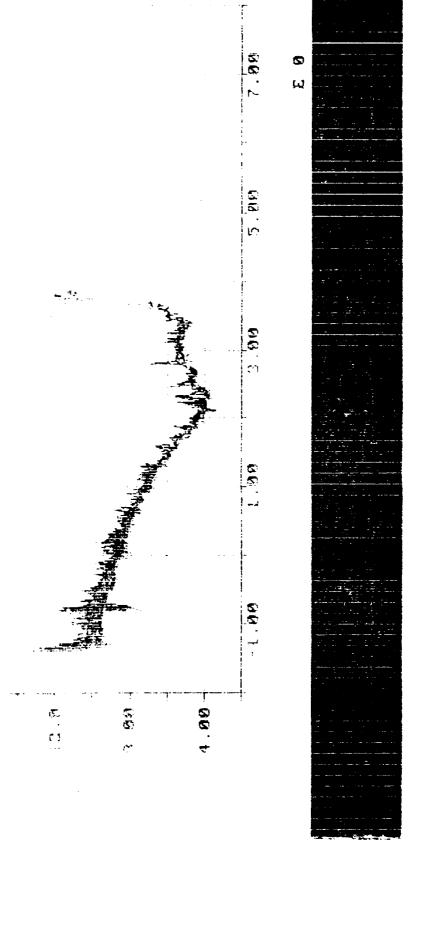
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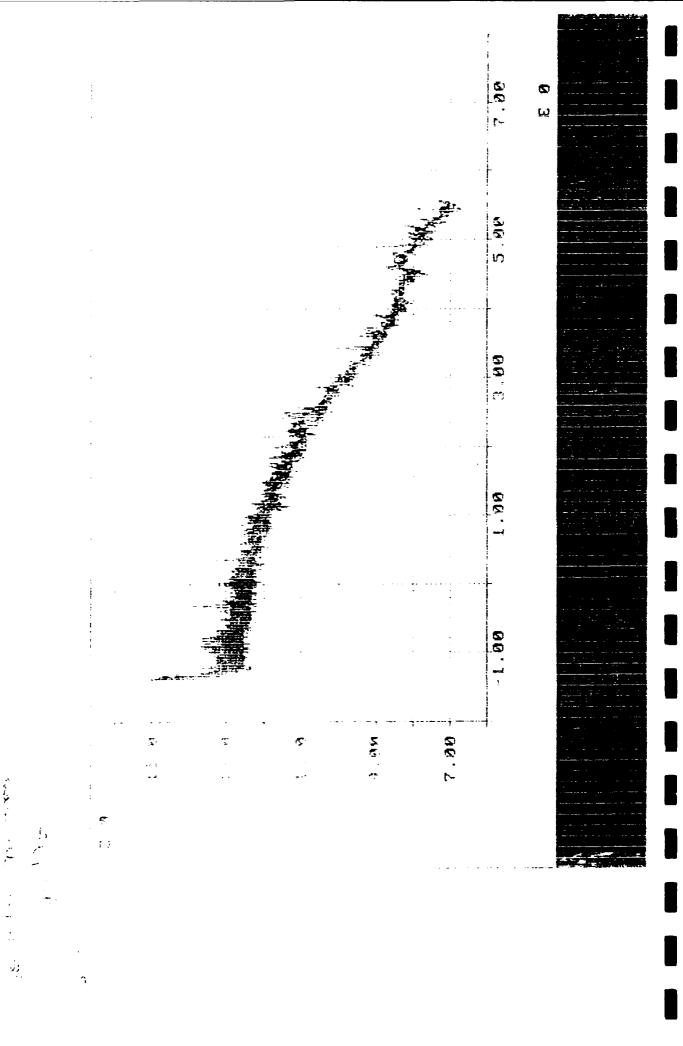






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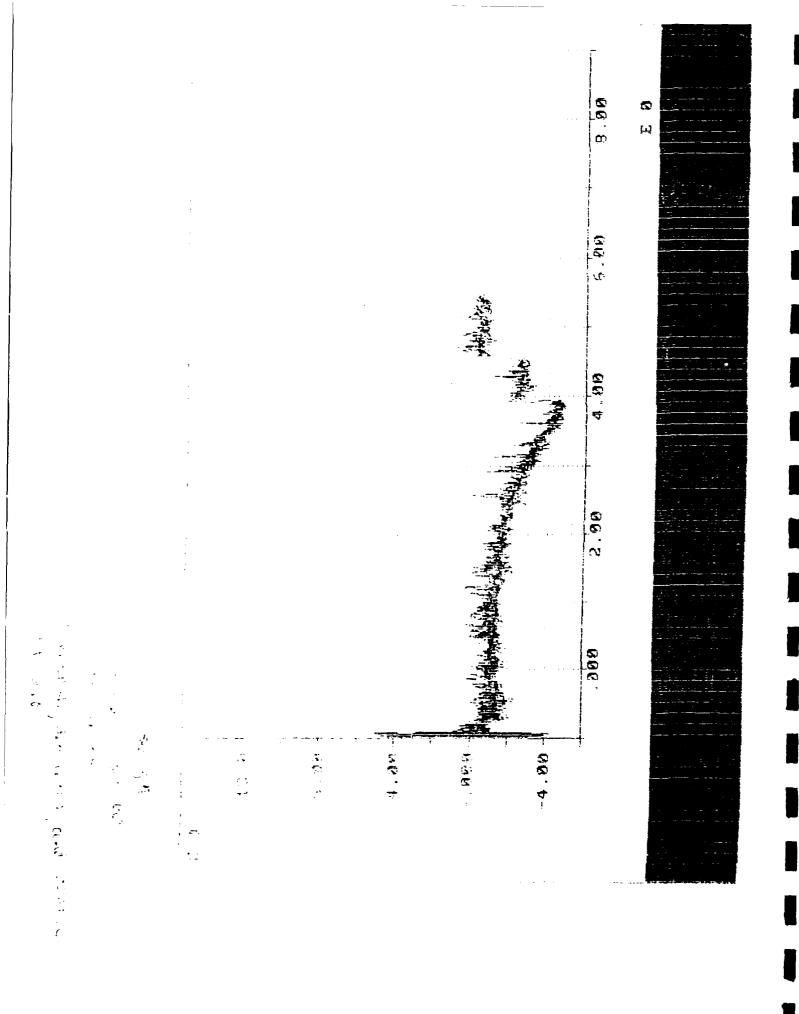
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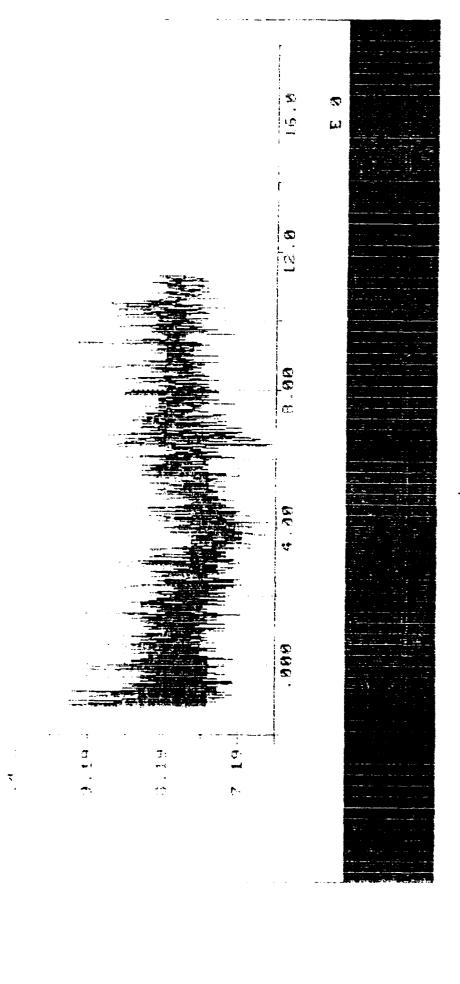
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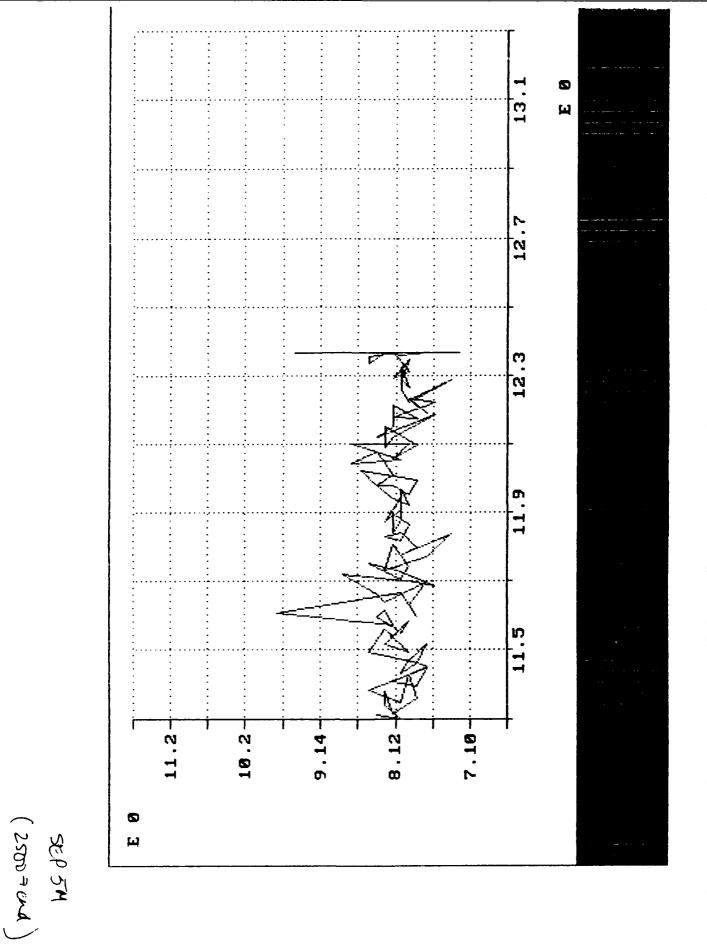
.**5** 

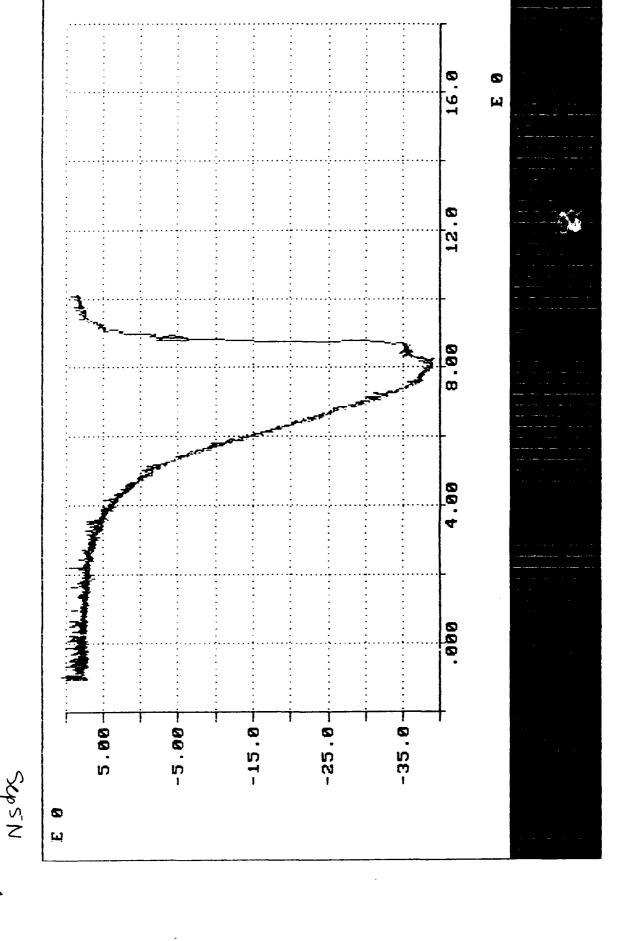
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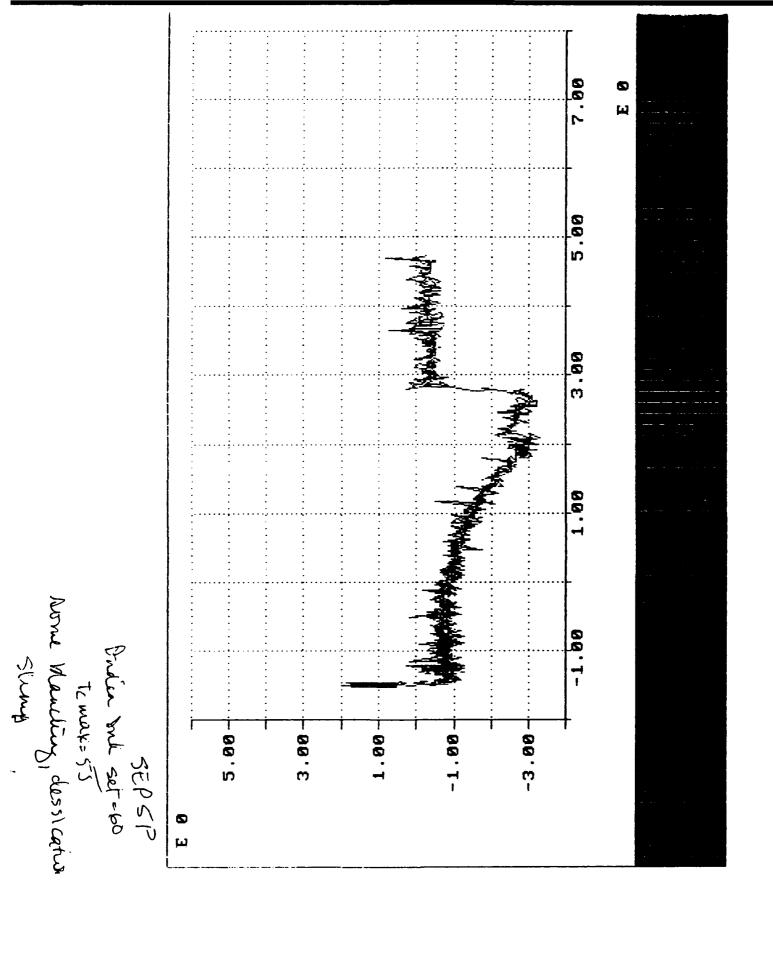
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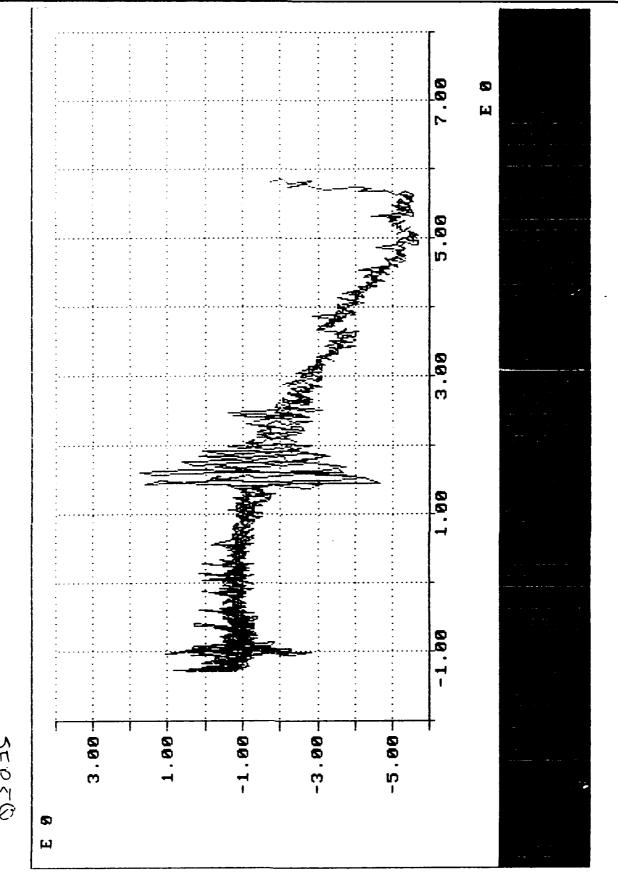
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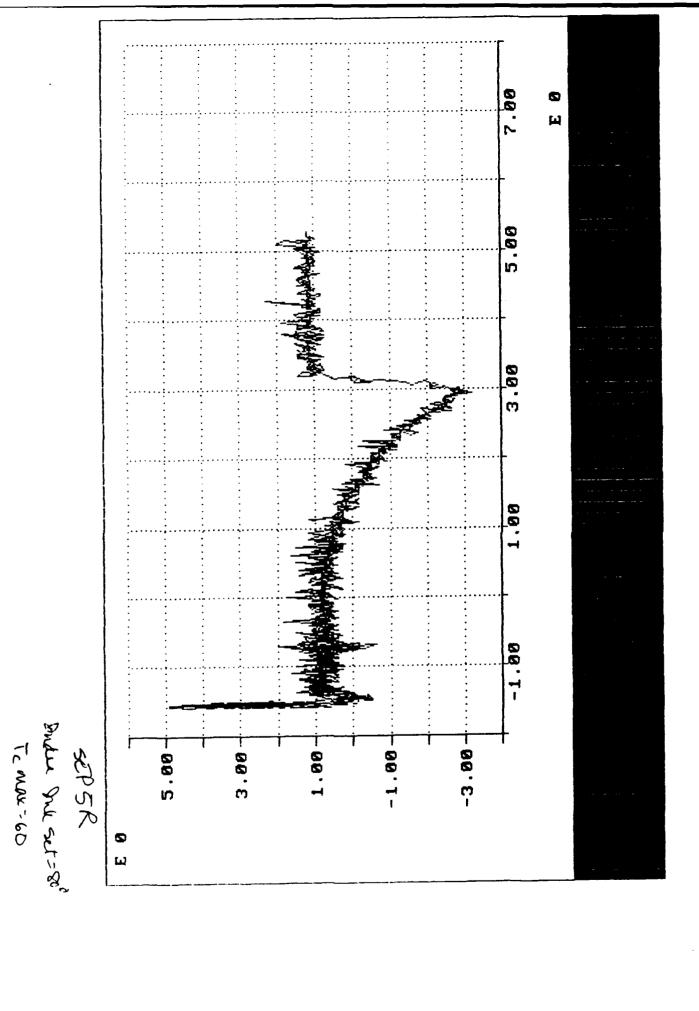
3.60 -2.00-- . 000 -Sep 50 E 8

India Juic controlled at 50 (main weld)

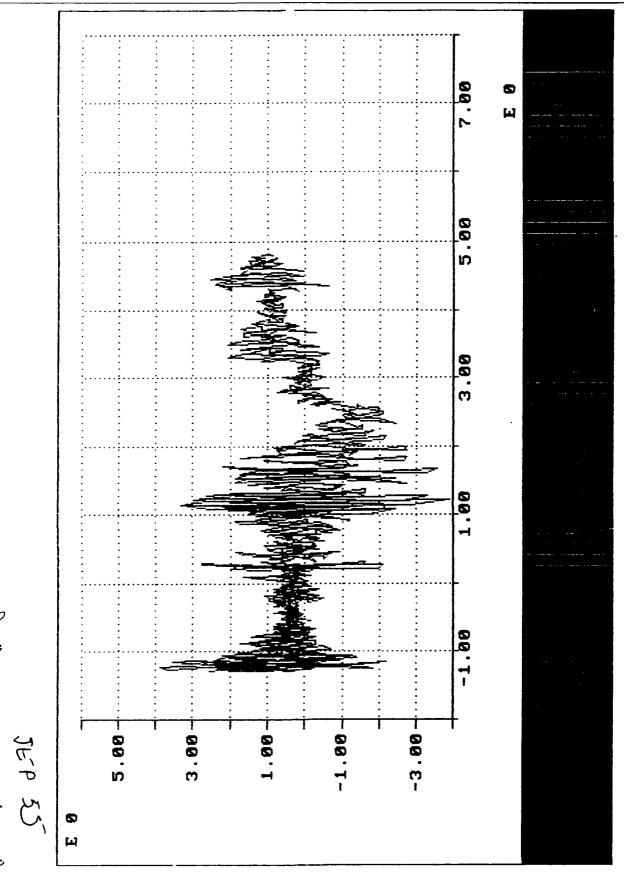




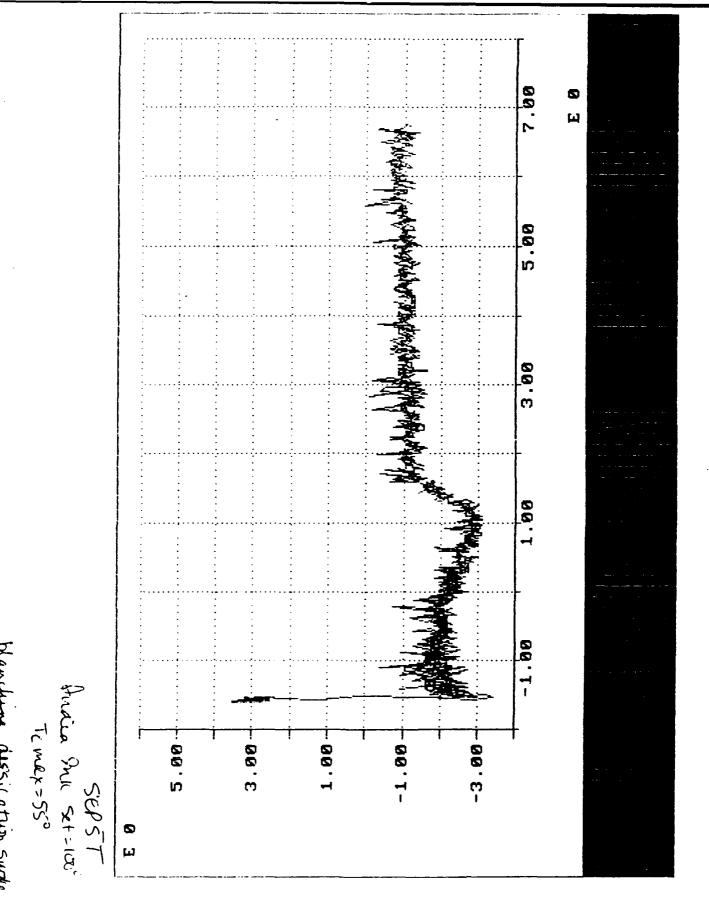
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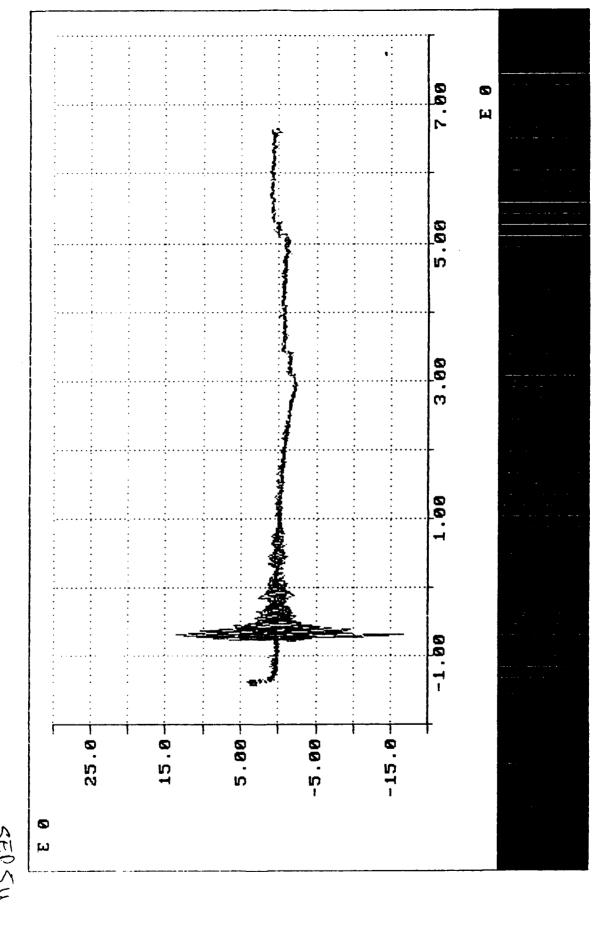
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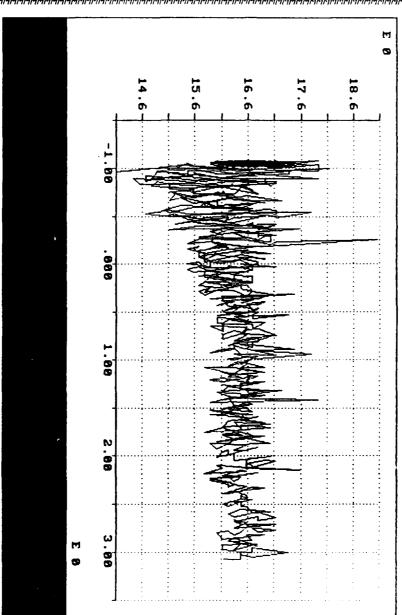
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   File Name: <C:SEF7U.USR:
                                    Quit/Continue: *
   8 Comments
                                      Subfiles (Total #: 3 )
: 1> INDIA INK
                                            Shape
                                  : Start#
                                                     #Repts :
: 2> CONTROLLED AT 60
: 3> SLIGHT DESSICATION
                                             2 = 256
                                      1
: 4> NOT VERY IMPRESSIVE
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: I/O Variable ( R - Z ): `
                                      1024 REAL
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                       2 : 256 (Max Length: 768 )
:
  Row (0=all): < 0 >
  Start Column: \langle |1 \rangle
  # of Columns: < 256 ^{\circ}
  Read/Write/Append/Scroll/Flot/Edit/List/New file/Quit: *
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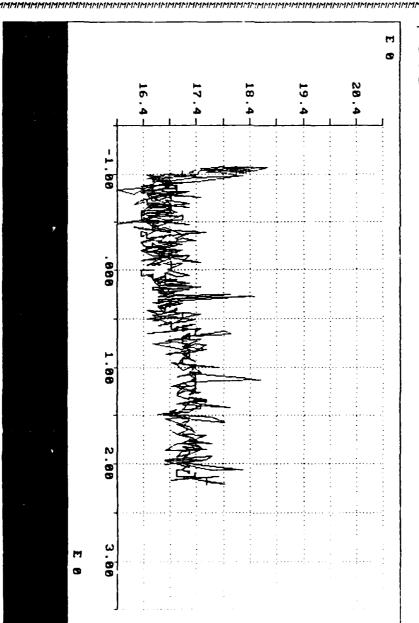


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File Name: (C:SEP7V.USK)
                                                                                                                                                                                                                                                           Quit/Continue: *
.
                                                                                                                                                                                                                                                                         Subfiles (fotal #: 2)
                        8 Comments
Shape #Kepts:
: 1> INDIA INK CONTROLLED AT 70
                                                                                                                                                                                                                                         : Start#
: 2> LITTLE VISUAL CHANGE
: 3> SOME SMOKE
: 4. NOT VERY STRONG
: 5
: 6
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: 8
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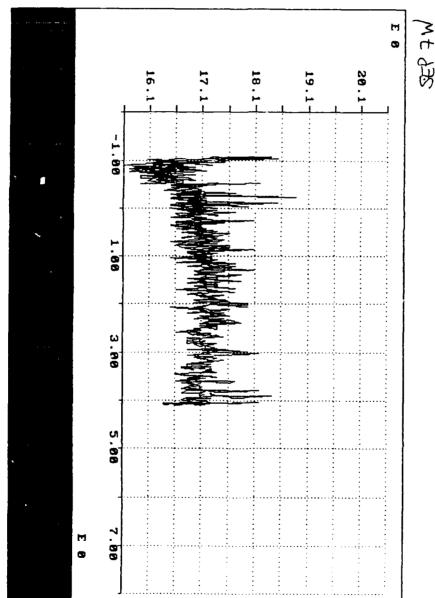
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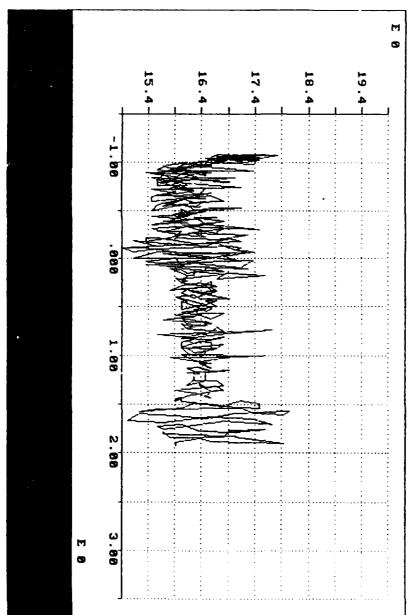


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File Name: C:SEF7W.USR
                          Quit/Continue: *
  8 Comments
                            Subtiles (Total #: 3 )
: 15 INDIA INF CONTROLLED AT 80 : Start#
                                Shape
2 + 256
                                       #Repts :
  SLIGHT DESSTRATION
                           1
  DID NOT LOOK VERY STRONG WHEN TEARING AFA:
: 4
. 5
: 6
: 7
: 8
: I/O Variable (R - Z):
                             512 REAL
 Subfile:
                 2 x 256 (Max Length: 768 )
 Row (0=all): < 0 >
 Start Column: < 1 >
 # of Columns: < 256 :
:
 Read/Write/Append/Scroll/Plot/Edit/List/New file/Quit: *
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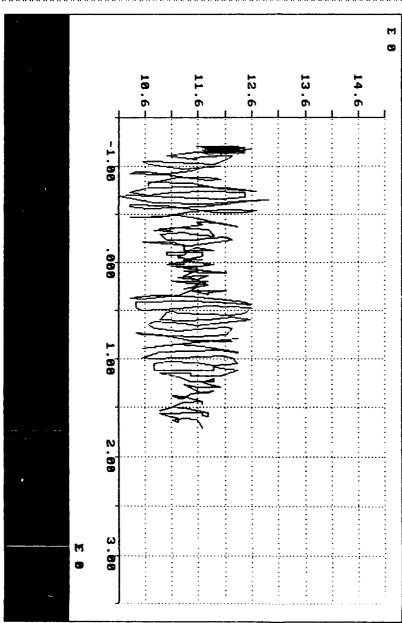
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       File Name: 'C:SEF'7X.USR'
                                     Quit/Continue: *
                                       Subfiles (lotal #: 2 )
       8 Comments
    : 1> INDIA INK LONGITUDINAL SECTION
                                  : Start#
                                            Shape
                                             J + 256
    2> CONTROLLED AT 70
                                    :
                                       1
    3> NO VISUAL CHANGES
   : 4' BROKE QUICKLY-DID NOT SEE A WELD
   : 5
1.
   : 6
   : 7
    : 8
    •
   : I/O Variable ( R - Z ):
                                       768 REAL
     Subfile:
                          2 :: 256 (Mar Length: 512 )
     Row (0=all): < 0
     Start Column: < 1
     # of Calumns: < 256
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     Read/Write/Append/Scroll/Plot/Edit/List/New file/Quit: *
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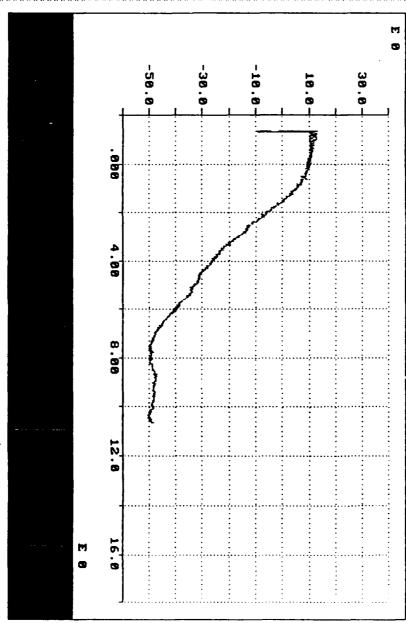


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Quit/Continue: *
                                            File Name: (B:SEF12A.USR
 :
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        Subfiles (Total #: 2 )
                                            8 Comments
 \mathbf{L}_{\mathbf{M}}^{\mathbf{M}}
 : 1> TWO STRIP CONTROL
                                                                                                                                                                                                                                                                                                                                                                                                                                          : Start#
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           #Repts :
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          Shape
: 2> SMALL APERTURE
: 3> SW
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 2 4 256
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           1
 : 4>
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                1024 REAL
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File Name: <B:SEP12B.USR>
                                                                                                                                                                          Quit/Continue: *
                8 Comments
                                                                                                                                                                                    Subfiles (Total #: 4 )
: 1> ONE STRIP CONTROL
                                                                                                                                                                                                                   Shape
                                                                                                                                                                                                                                                             #Repts :
                                                                                                                                                                  : Start#
: 2> NO SLIPPAGE
                                                                                                                                                                                                                    2 × 256
                                                                                                                                                                                     1
: 3>
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: I/O Variable (R - Z):
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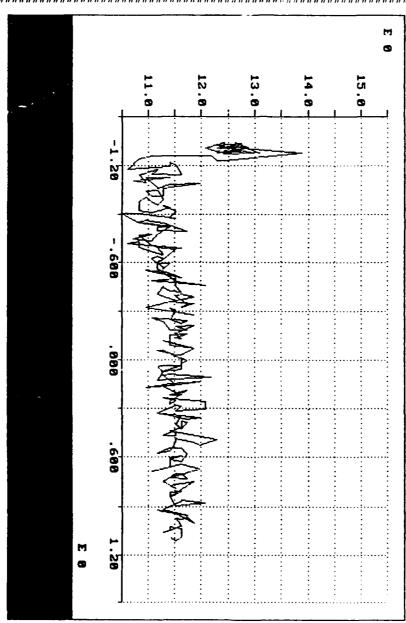


```
File Name: <B:SEP12C.USR:
                           Quit/Continue: *
  8 Comments
                            Subfiles (Total #: 1 )
: 1> INDIA INK UNWELDED CONTROL
                         : Start#
                                 Shape
                                        #Repts :
: 2>
: 3>
  Small Aperture
                                  2 % 256
                          :
                             1
: 4>
: 5>
: 7>
: 8>
:
 I/O Variable (R - Z):
                             1024 REAL
                  2 x 256 (Max Length: 256 )
 Subfile:
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File Name: <B:SEF12D.USR>
                                Quit/Continue: *
      8 Comments
                                  Subfiles (lotal #: 2 )
Ĺ
   : 1> INDIA INK CONTROLLED AT 50
                              : Start#
                                       Shape
                                              #hepts :
   : 2> LOOKS LIKE A WELD
                                       2 8 256
   : 3> NO SMOKE
   : 4> NO BLANCHING
   : 5> WEAK WELD
: 6> Small Aperture
      SW
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                                  256 DEAL
                      2 % 256 (Max Length: 512 )
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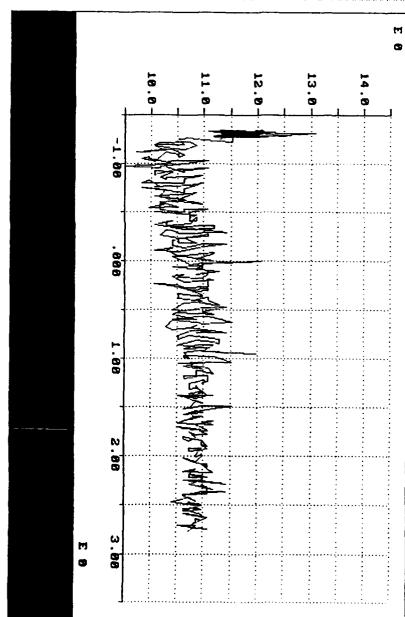
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File Name: <B:SEP12E.USR:
                            Quit/Continue: *
                              Subfiles (Total #: 3 )
: 1: INDIA INK SMALL AFERTURE
                           : Start#
                                   Shape
                                          #Repts :
: 25 CONTROLLED AT 60
: 35 NO VISUAL CHANGES
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: 4> WEAF WELD
: 5> Small Aperture : 6> SW
: 7>
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                  2 x 256 (Max Length: 768 )
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 Start Column: < 1
 # of Columns: < 256 >
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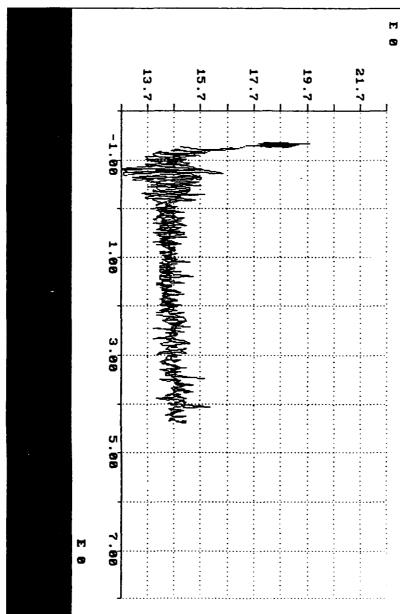
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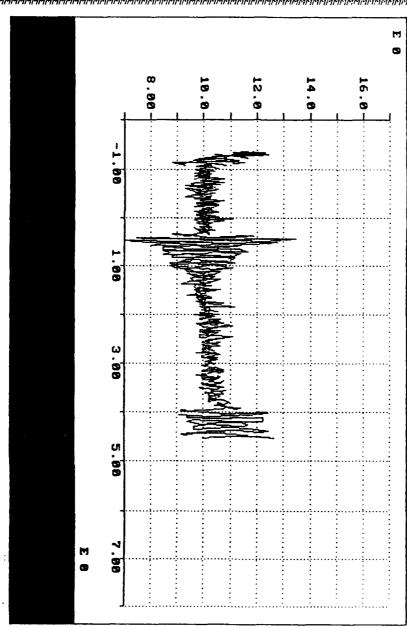
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: 1> INDIA INK CONTROLLED AT 70
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: 43 MEDIUM STRONG WELD
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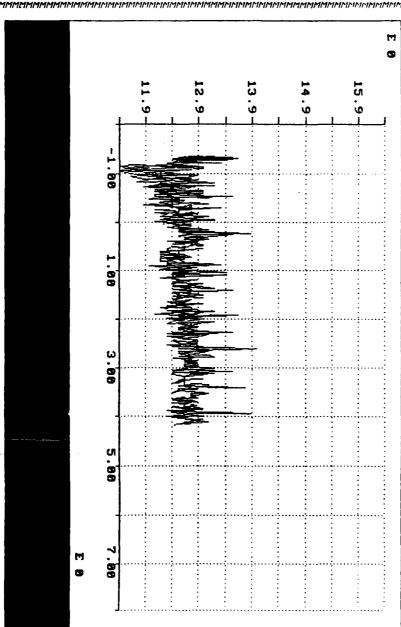
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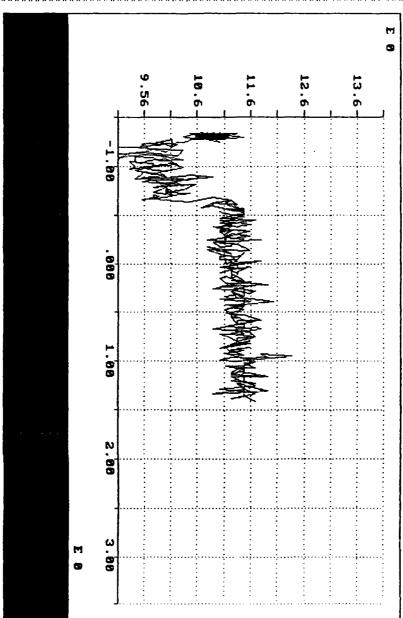


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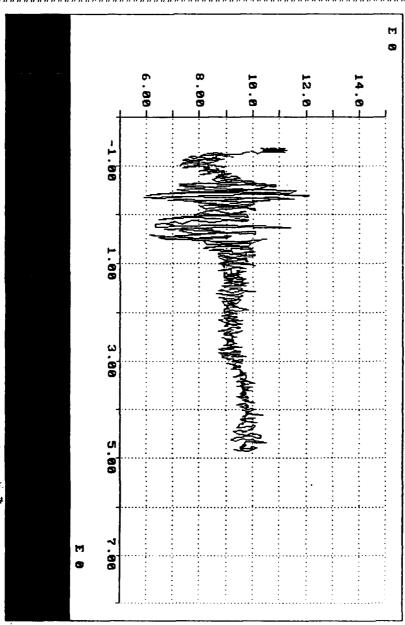


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                   3> SMALL AFERTURE
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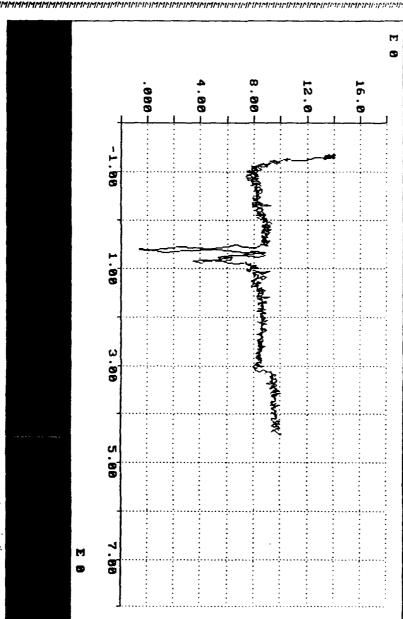


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: 4> STILL LOOKS WEAR
: 5> Small Aperture : 6> SW
: 7:
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: 3> SMELLS COOKED
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: 6> WEAK WELD
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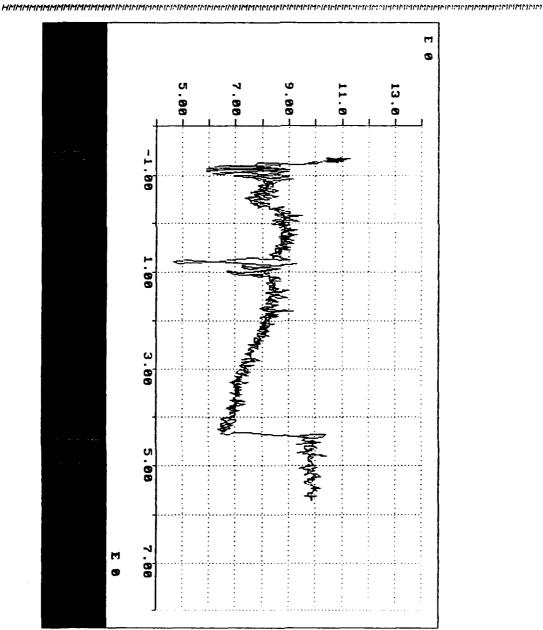
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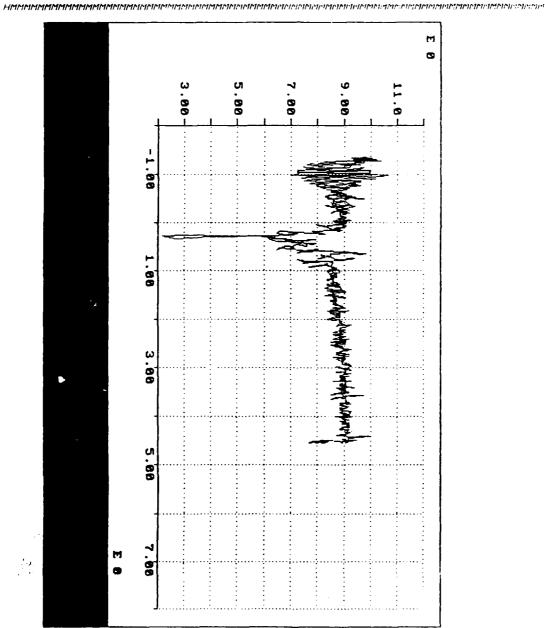
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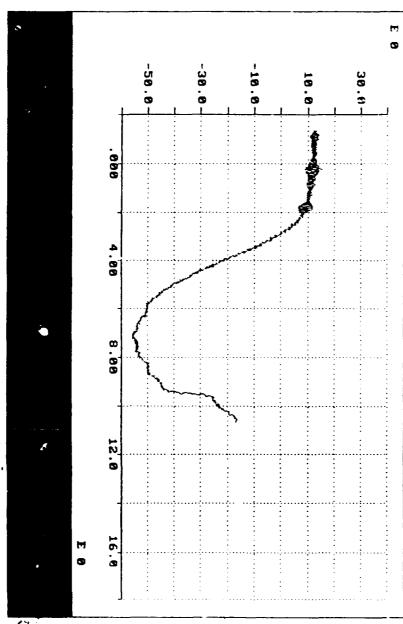
 Start Columns:
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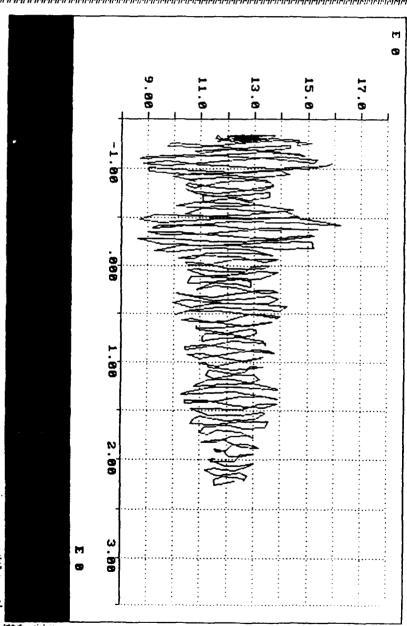
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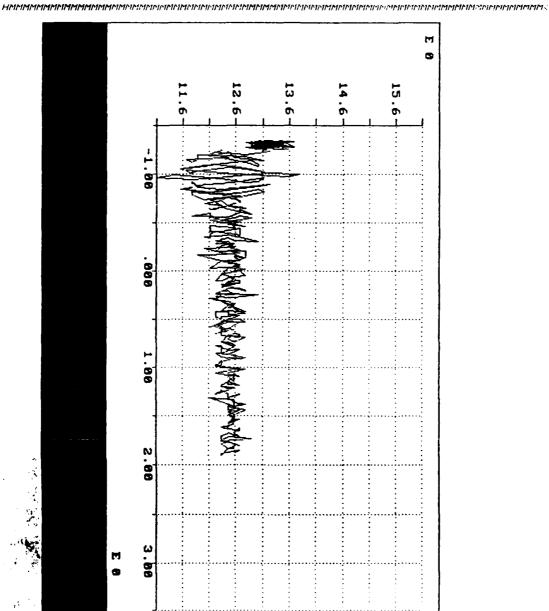
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                                             Total #: 4 )
: 15 ONE STRIP CONTROL UNWELDED
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: 2 LARGE APERTURE
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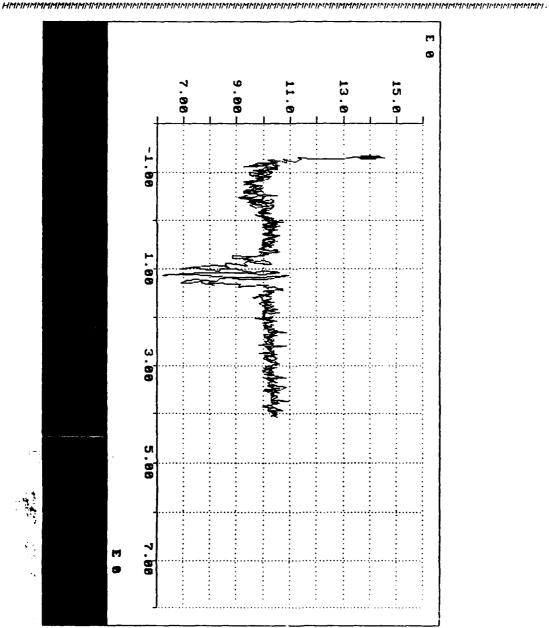


Interpretable interpretable interpretable in the individual control of the individual interpretable in the individual ind File Name: <B:SEP120.USF Quat/Fontinge: \* 8 Comments Subfile: : lotal #: 2 ) TWO STRIF INDIA INF UNWELDED CONTROL 1. : Start# shape larg aperture : 23 . 53 : 63 : 7> 8 : I/O Variable ( R - Z ): 512 PEAL Subfile: 2 x 256 (Mar Length: 512 ) Row (0=all): < 0 : Start Column: < 1 : # of Columns: 3 256 5 Read/Write/Append/Scroll/Flot/Edit/List/New file/Quit: \* :



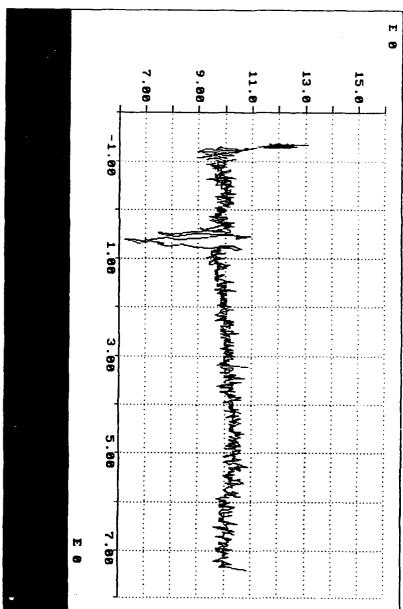
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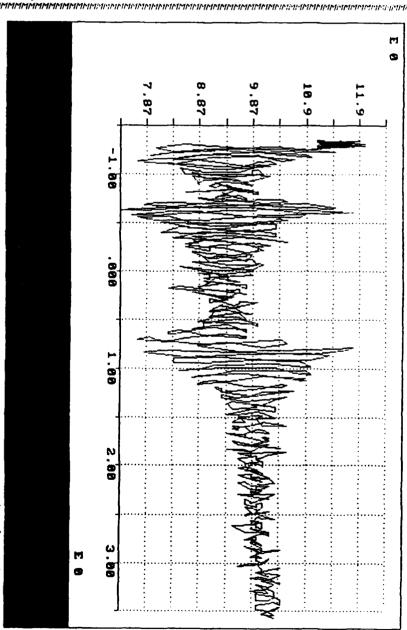
Read/Write/Append/Scroll/Flot/Edit/List/New file/Quit: \*



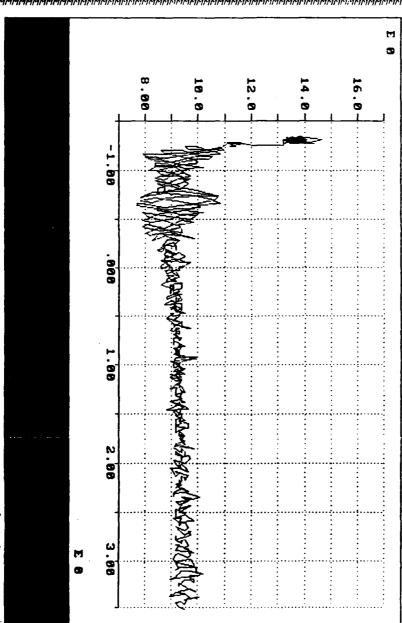
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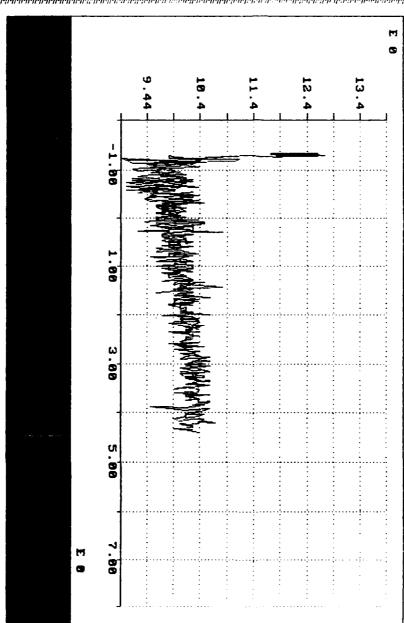




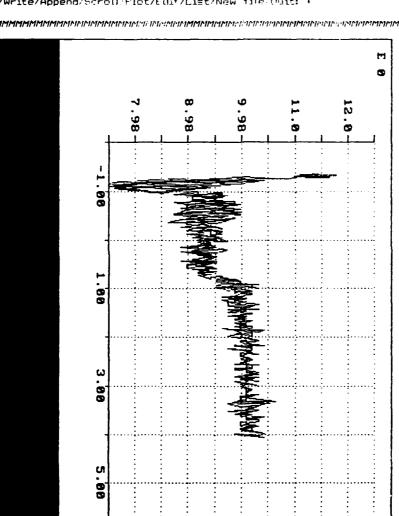
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                8 Comments
: 1> INDIA INK CONTROLLED AT 70
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: 2> LARGE APERTURE
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: 35 MINIMAL DESSICATION
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I/O Variable ( R - Z ):
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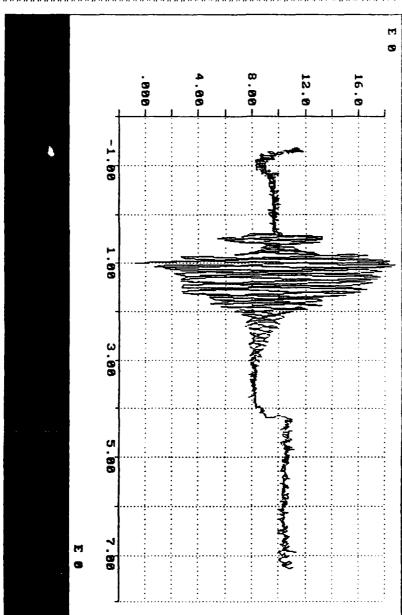


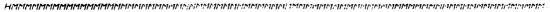


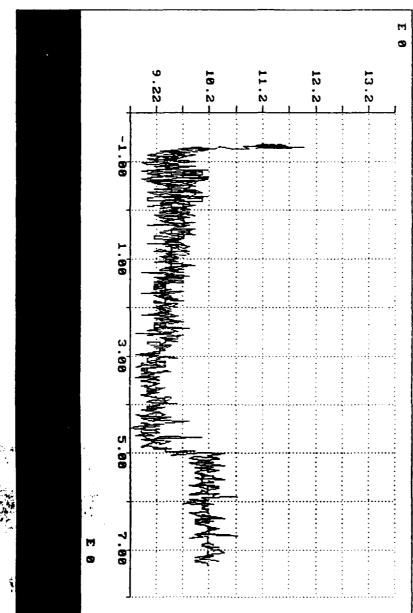
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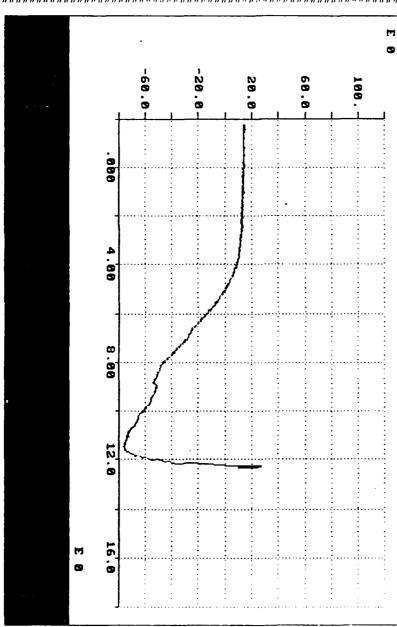
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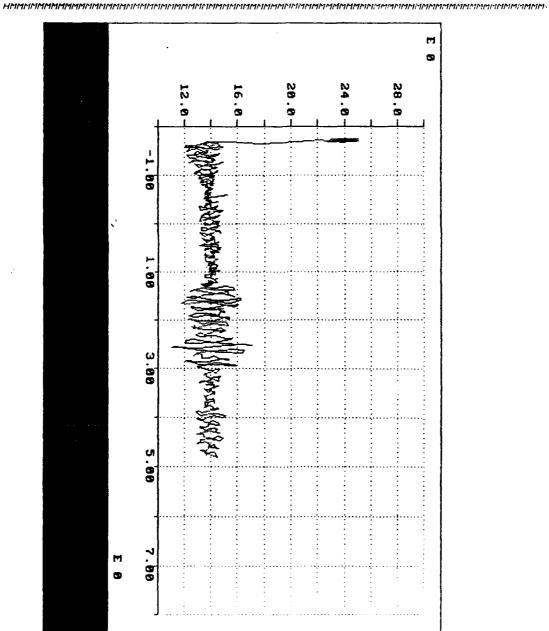




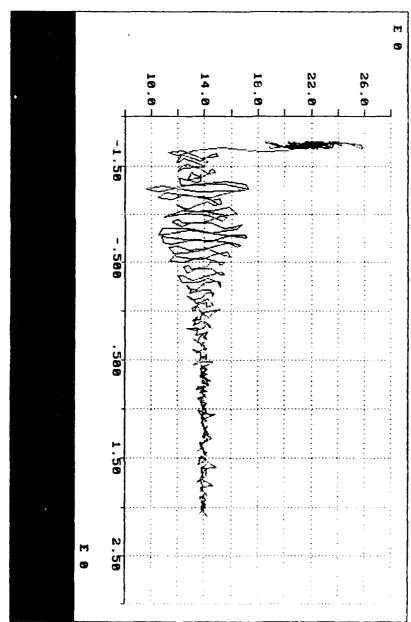
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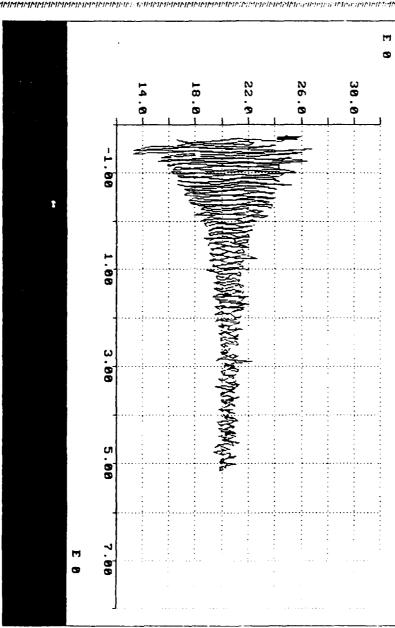
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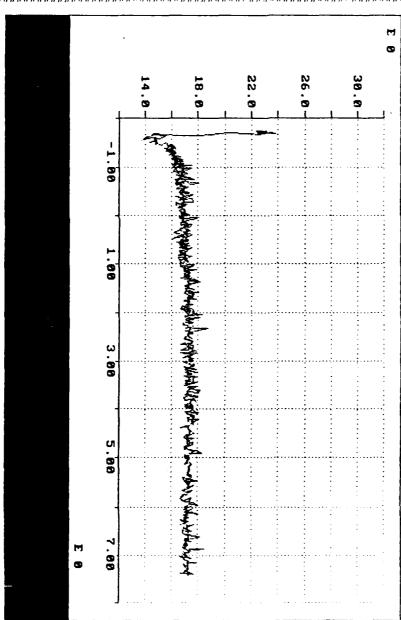
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: 1 NO SMOKE
: 4 NO OBVIOUS WELL OFF
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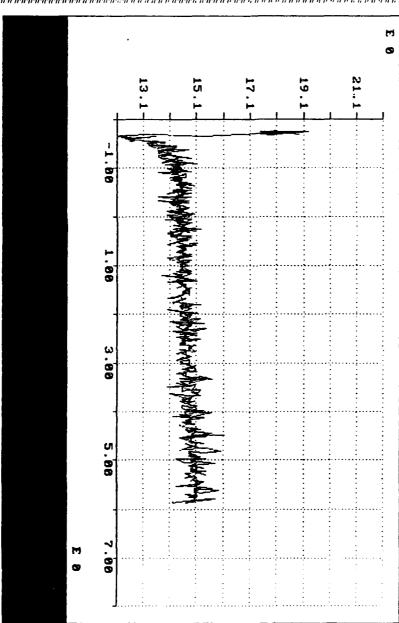


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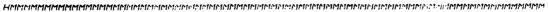


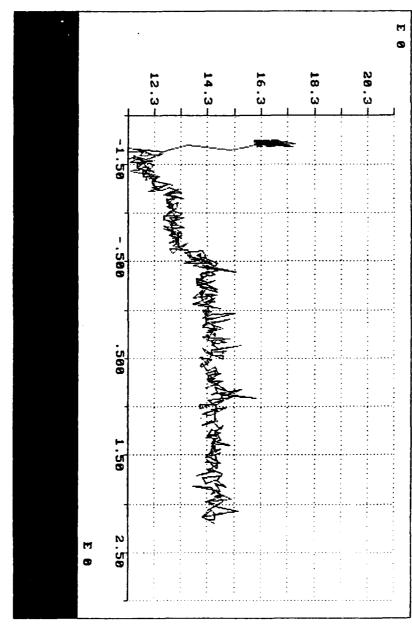
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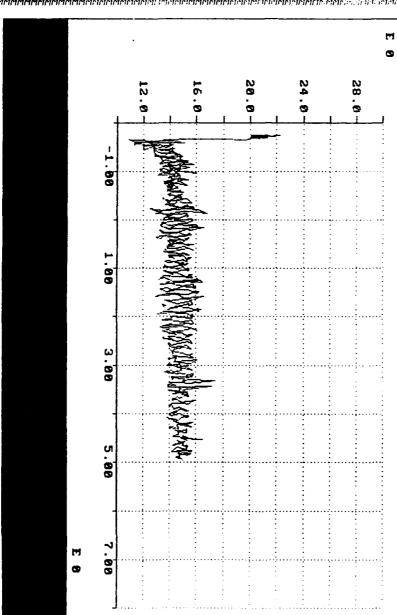


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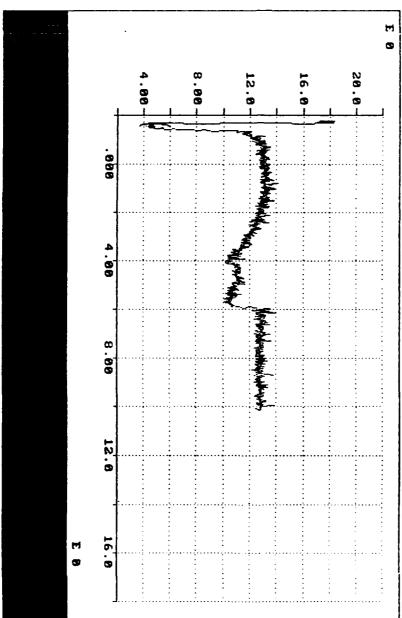




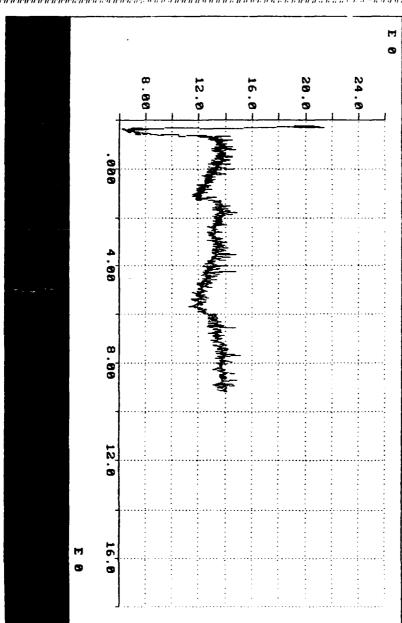
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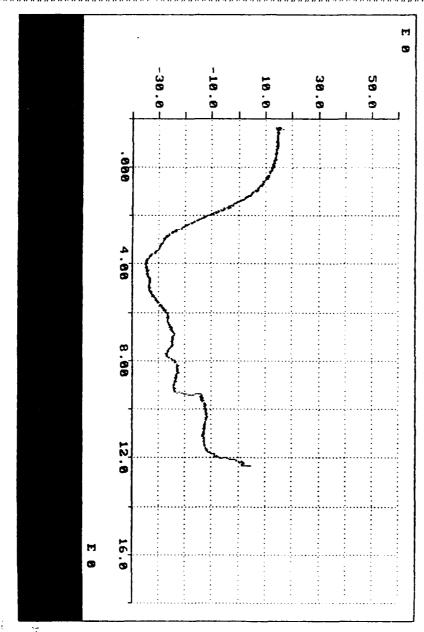
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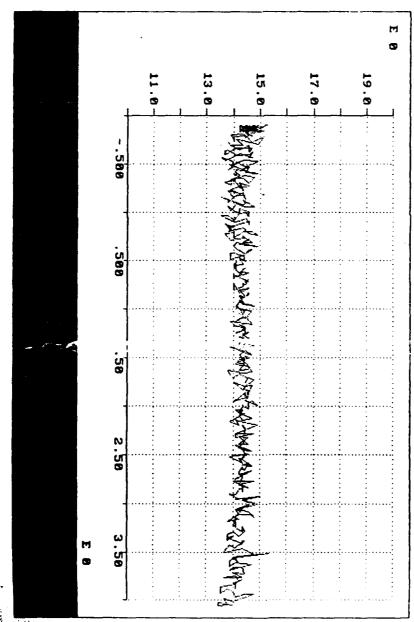
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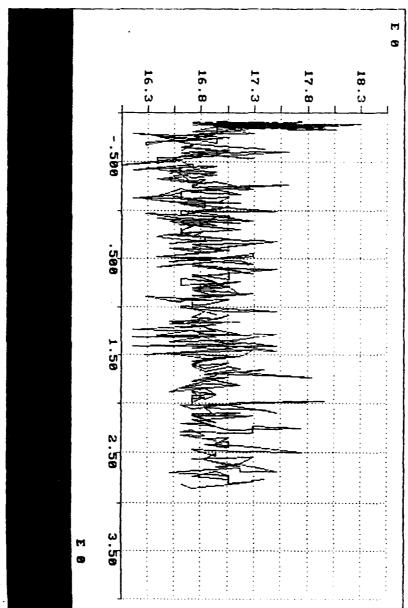
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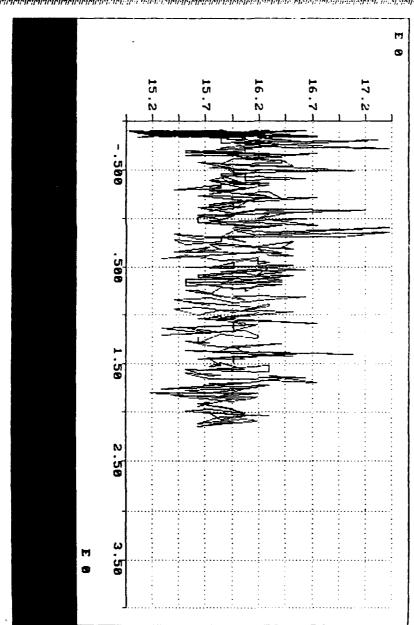
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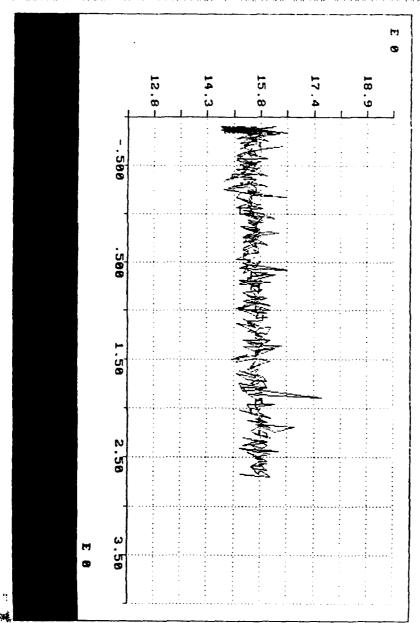


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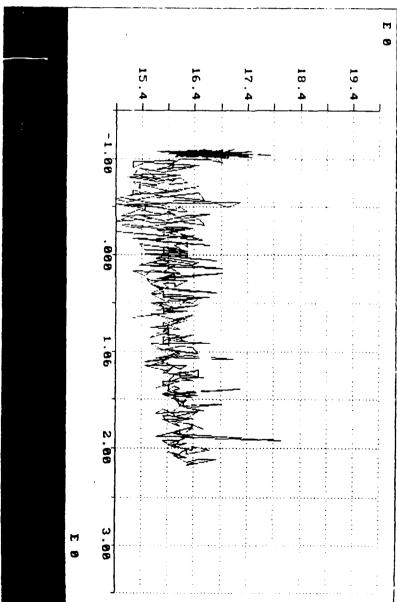
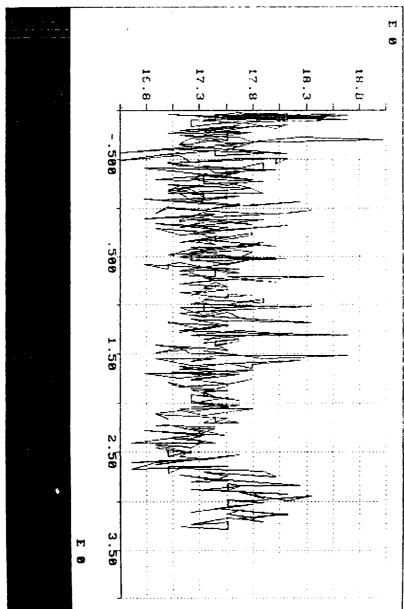


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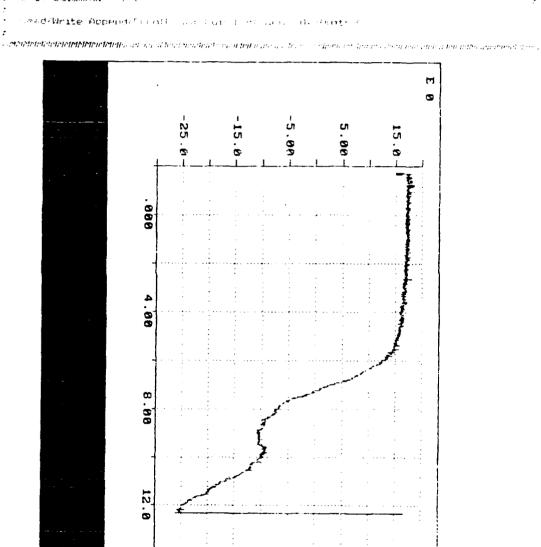
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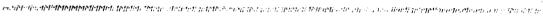


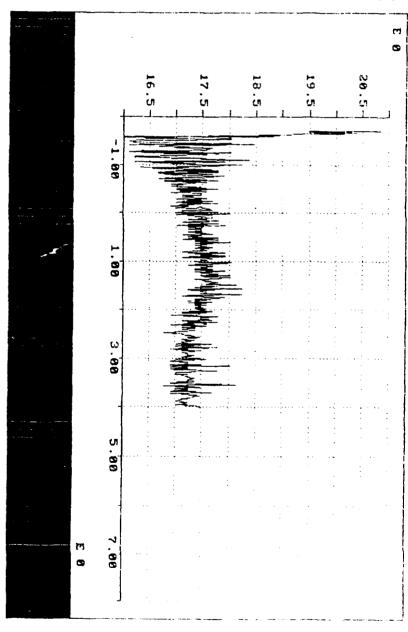
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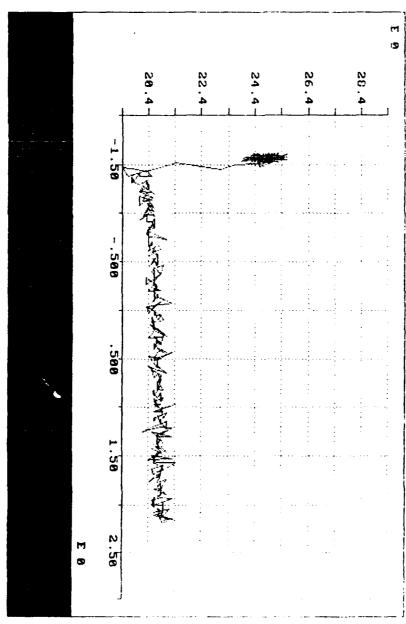
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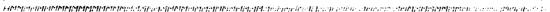


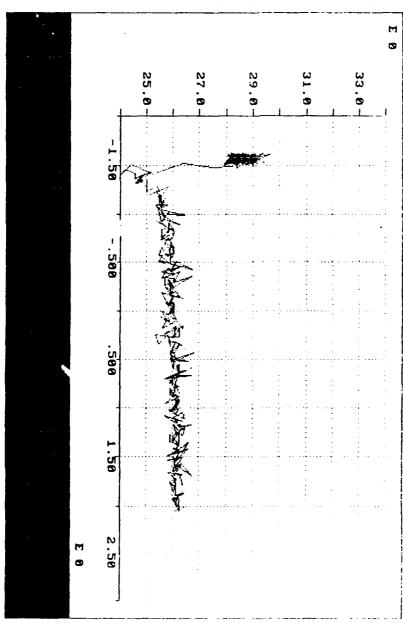


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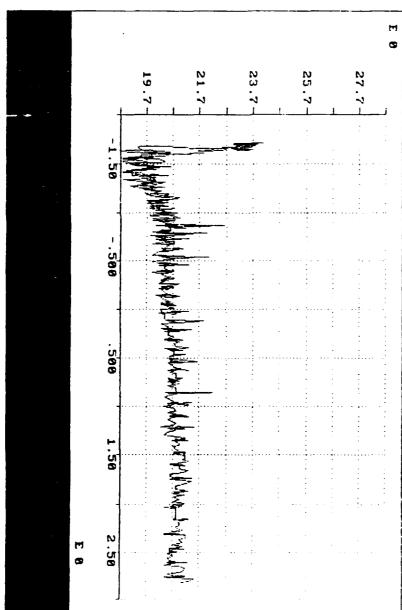
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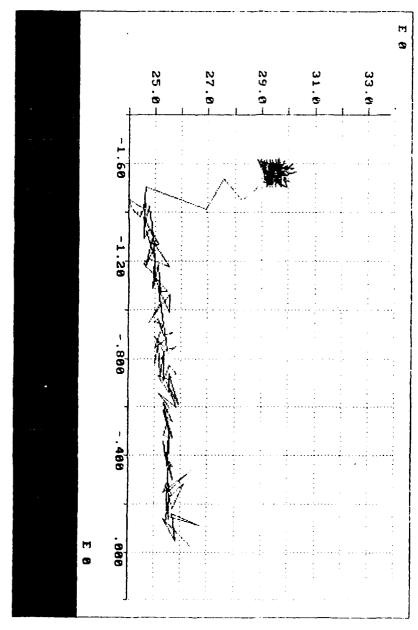
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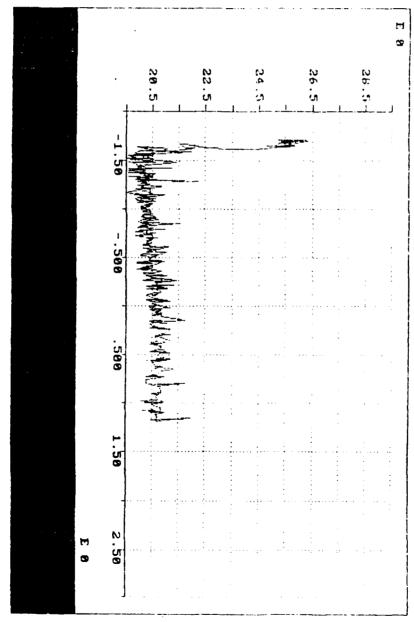


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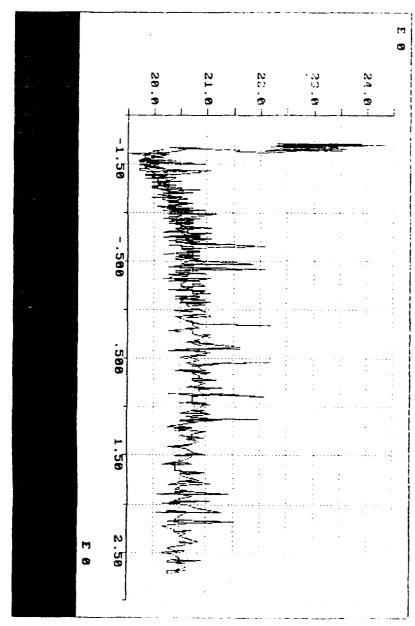
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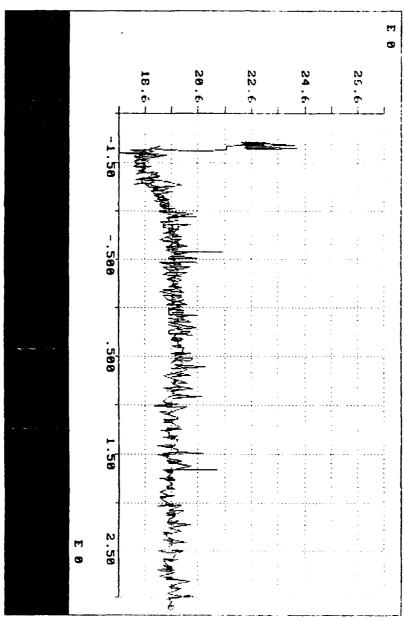
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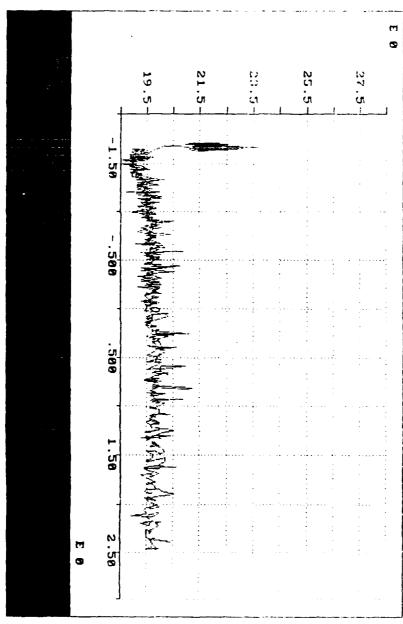


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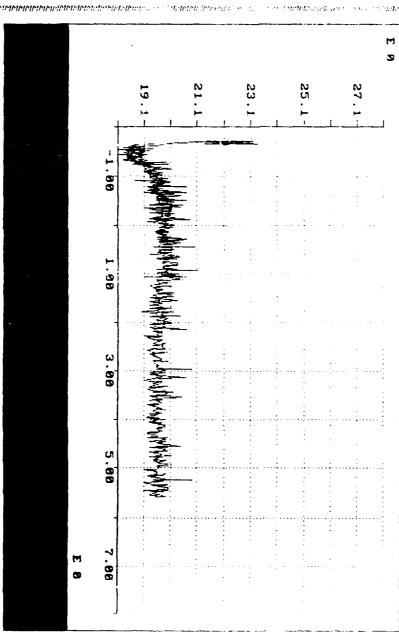
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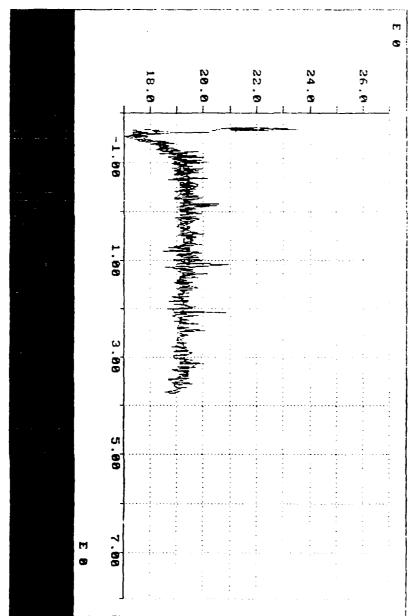


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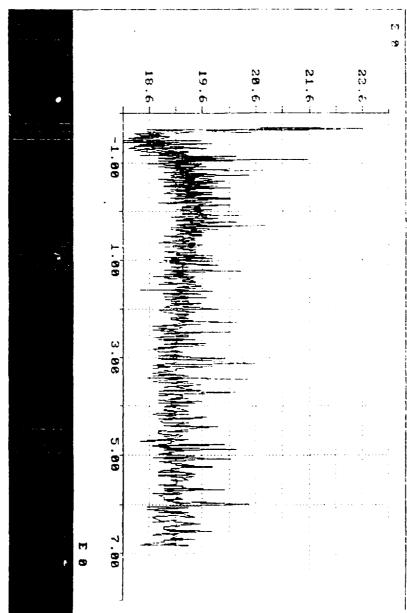
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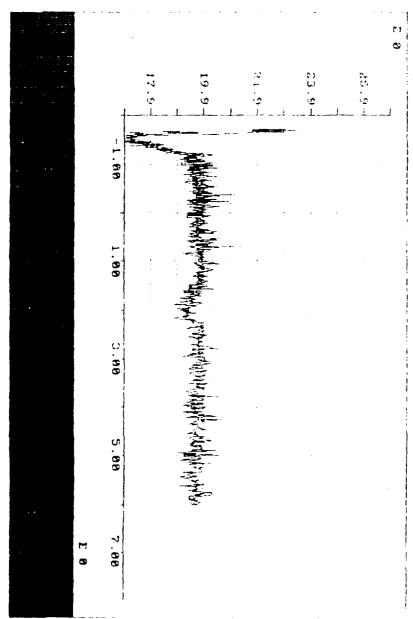
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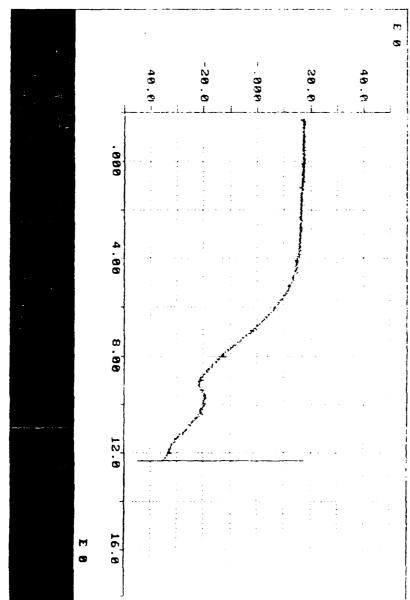
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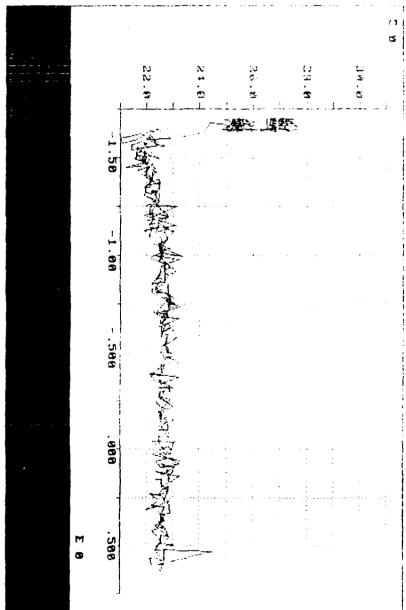
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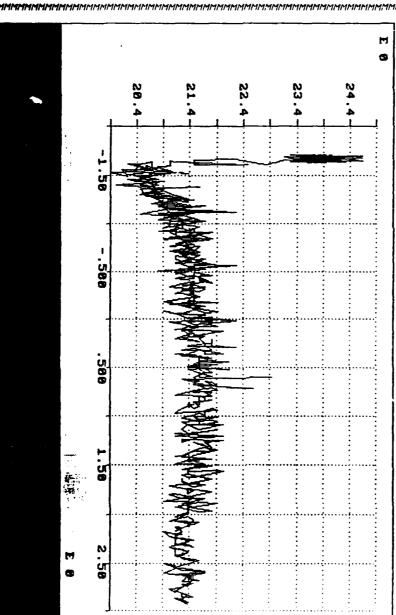
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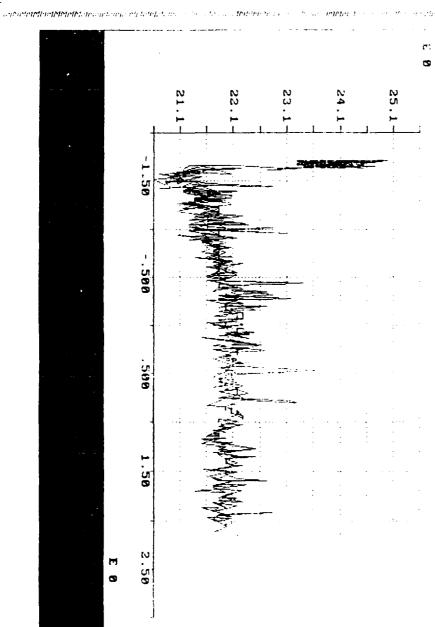
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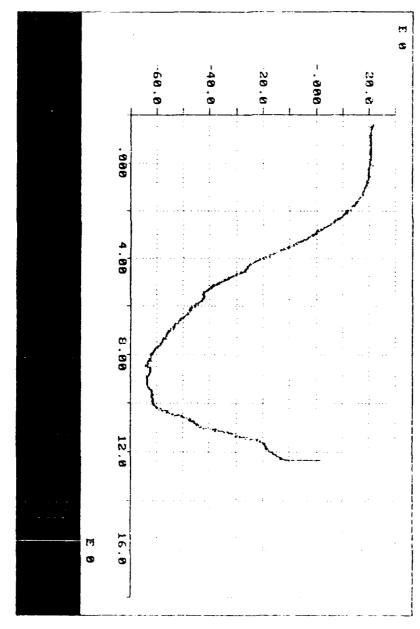
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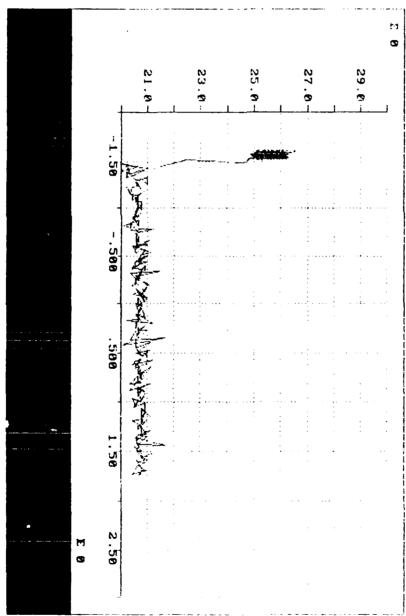


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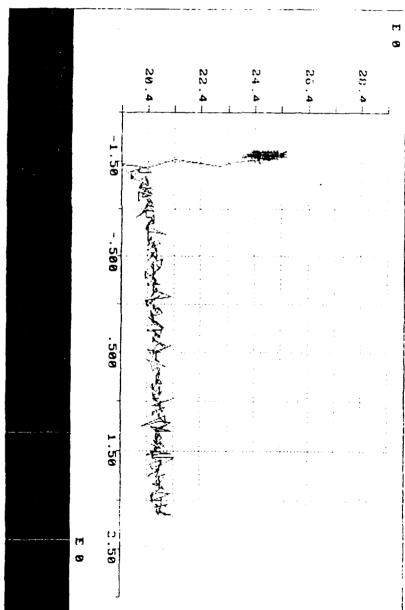
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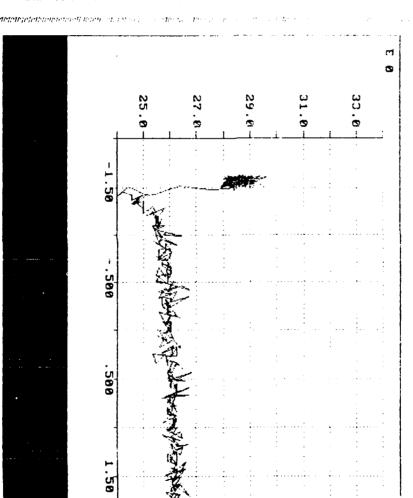
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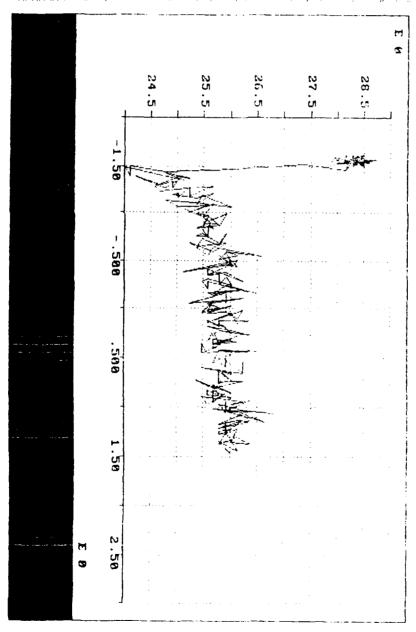
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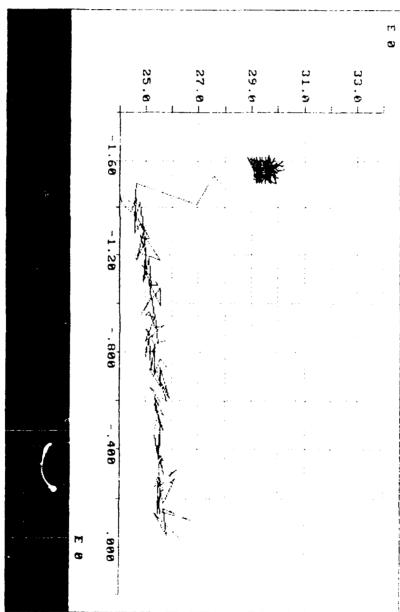
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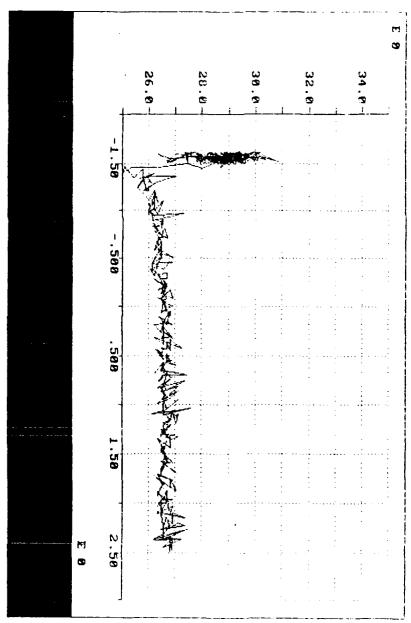
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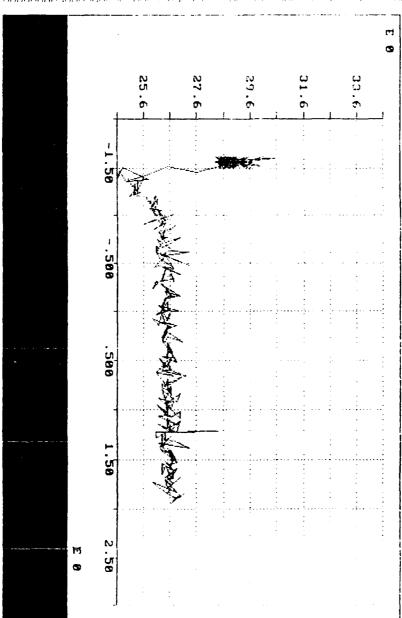


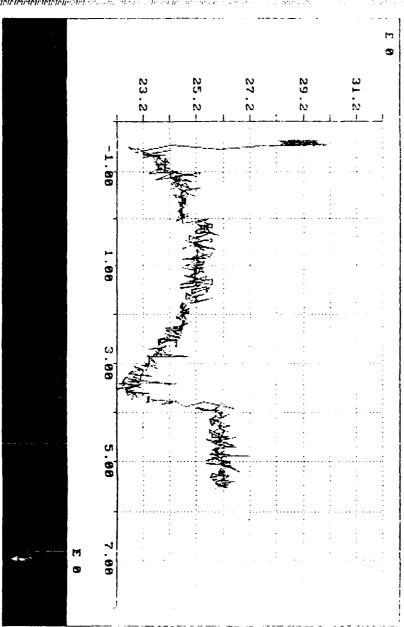


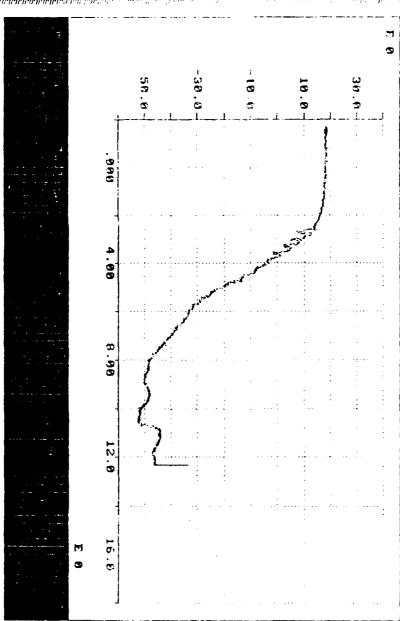
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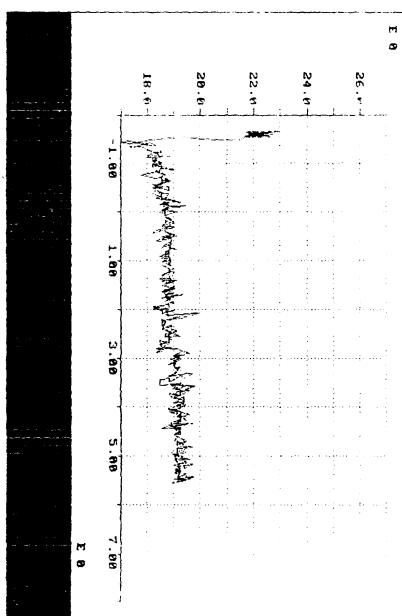




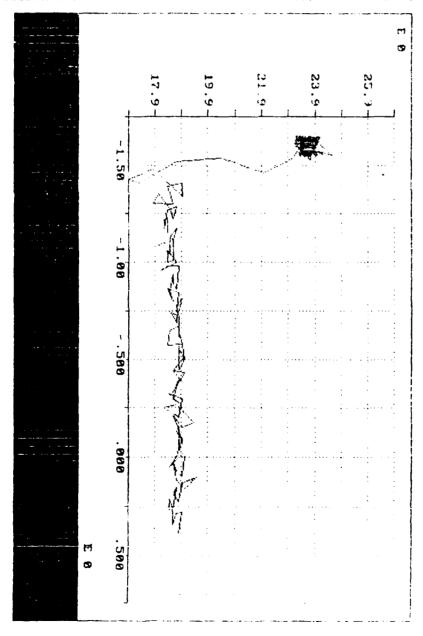


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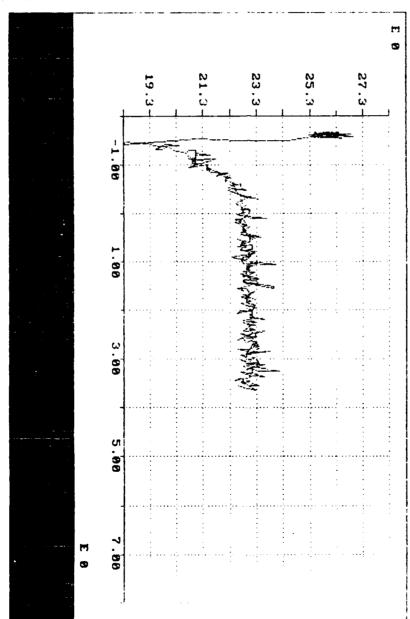
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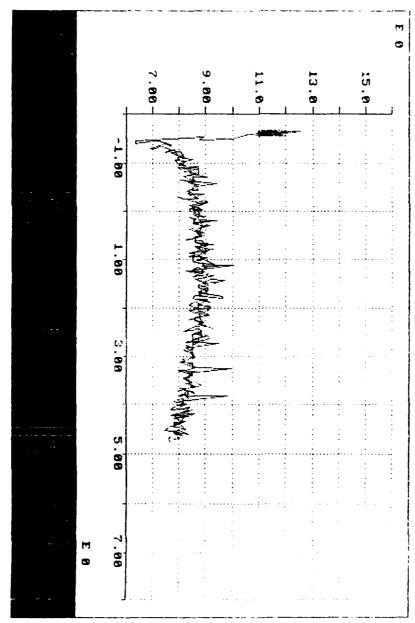
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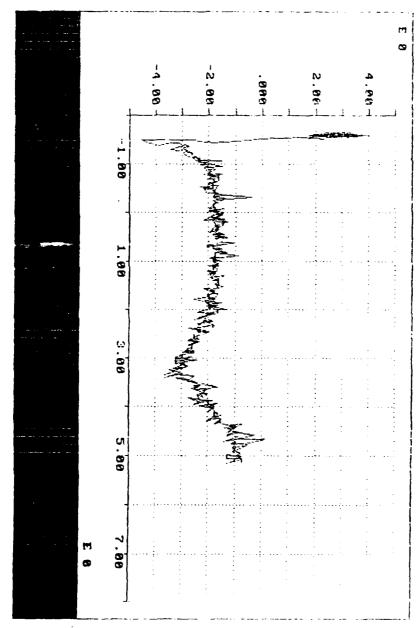
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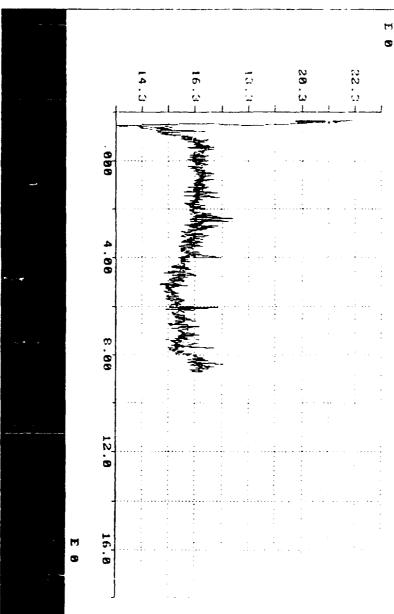
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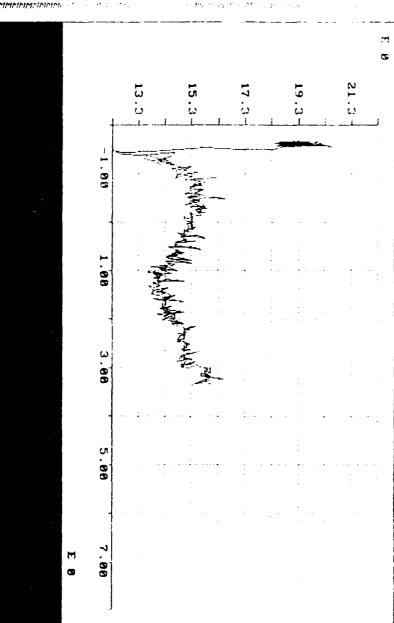
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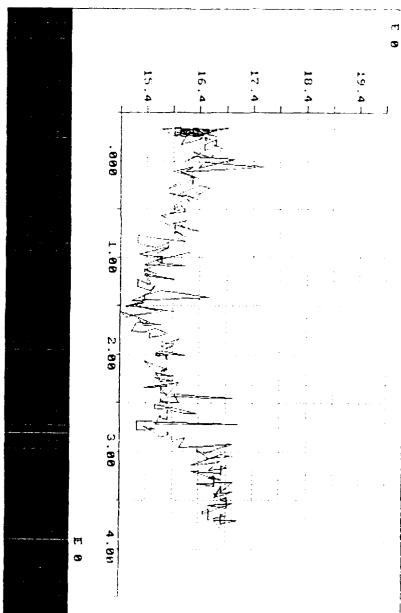


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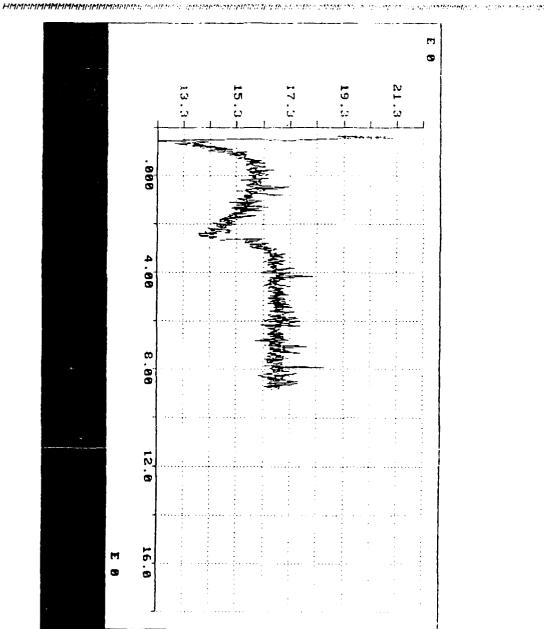


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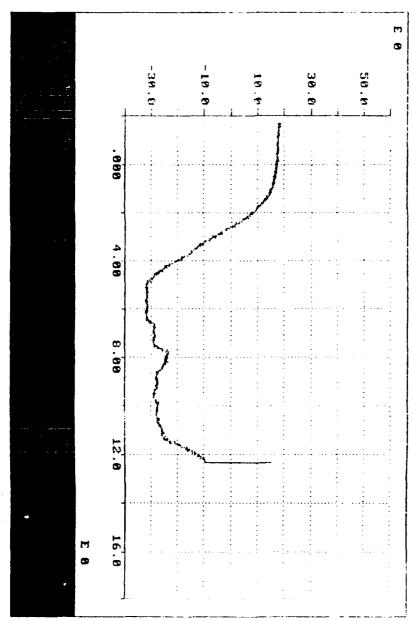
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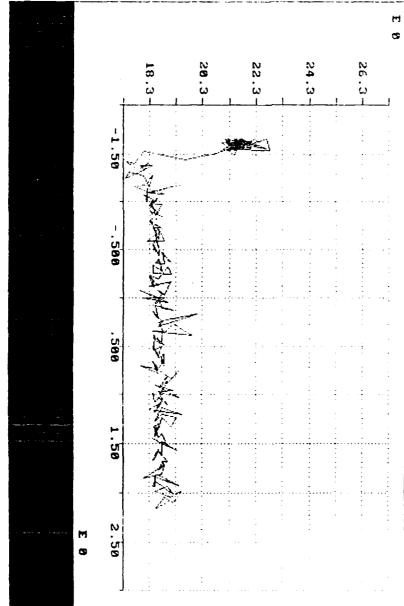


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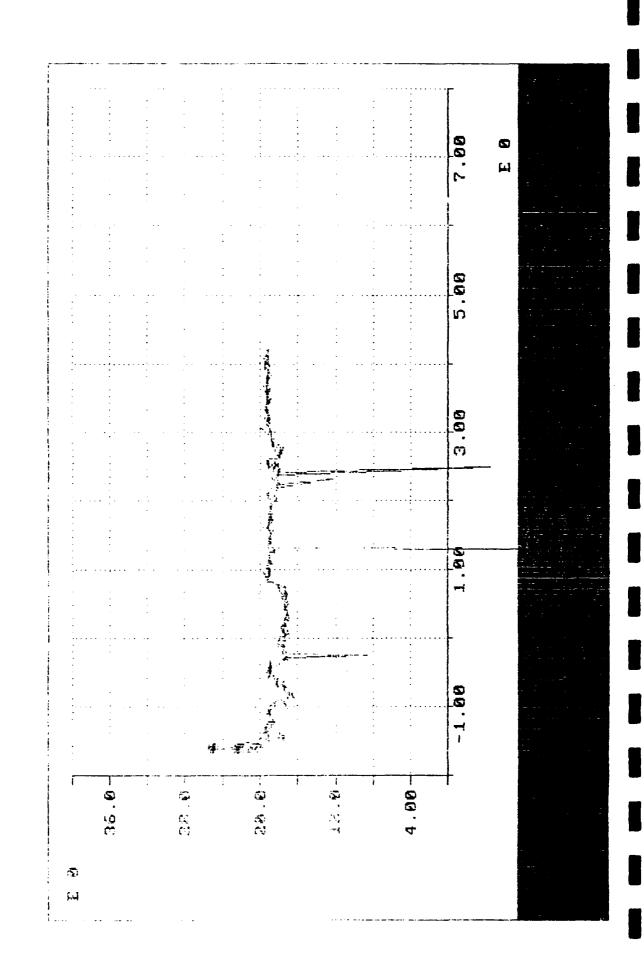


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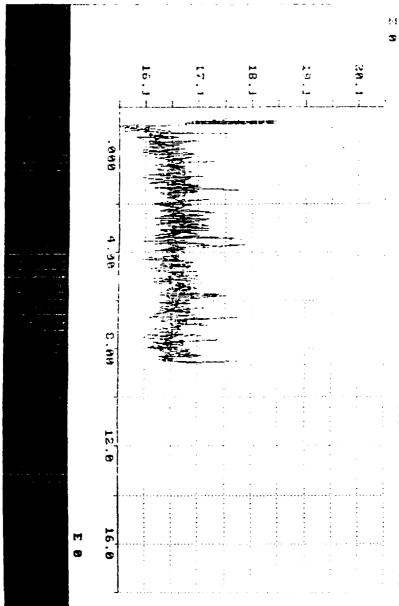


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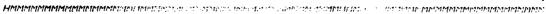
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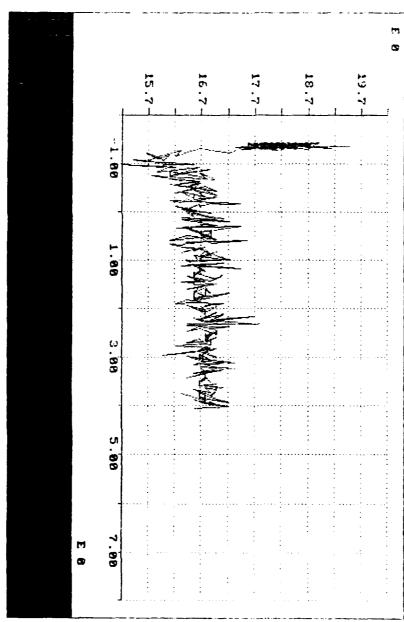
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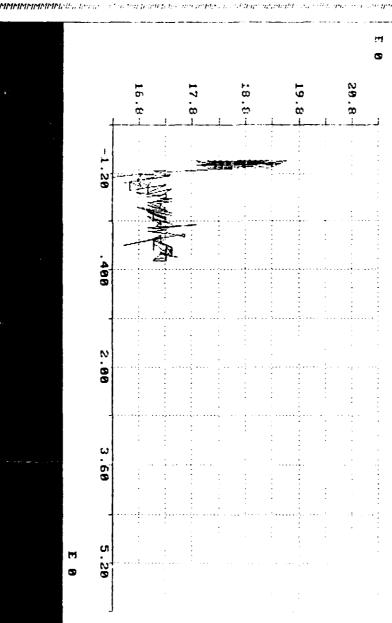
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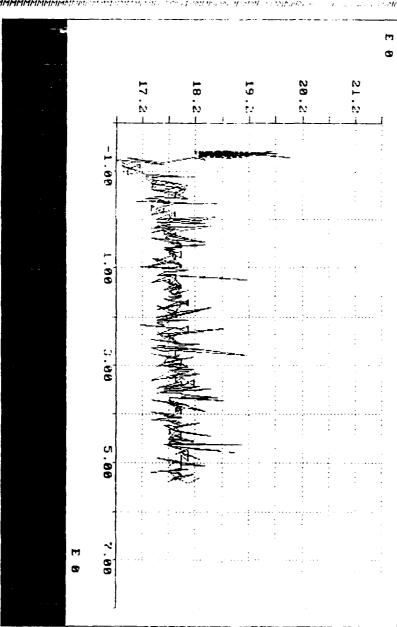




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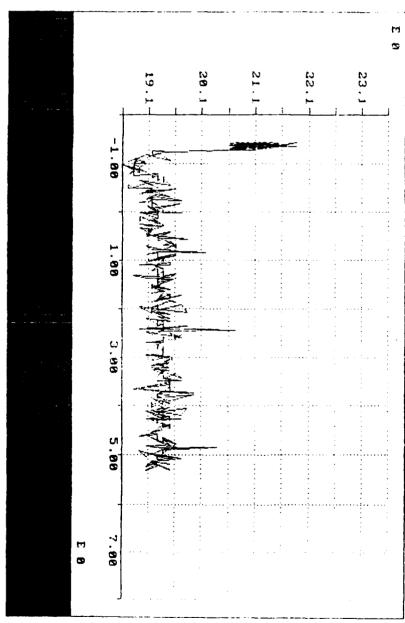


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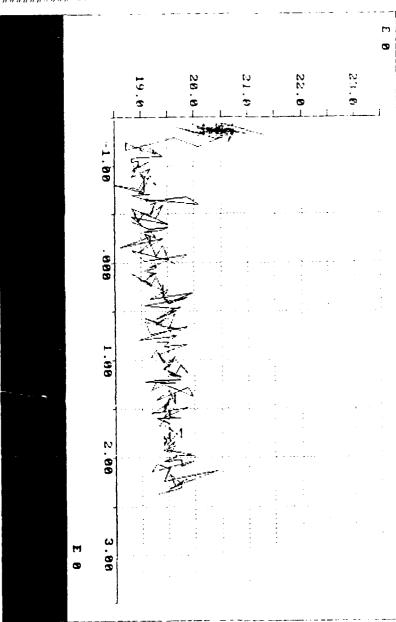


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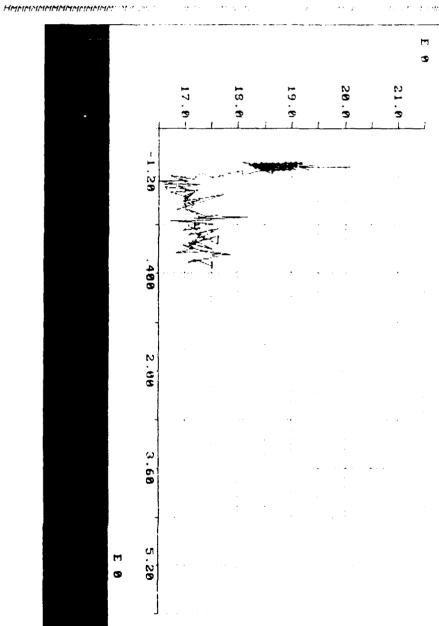
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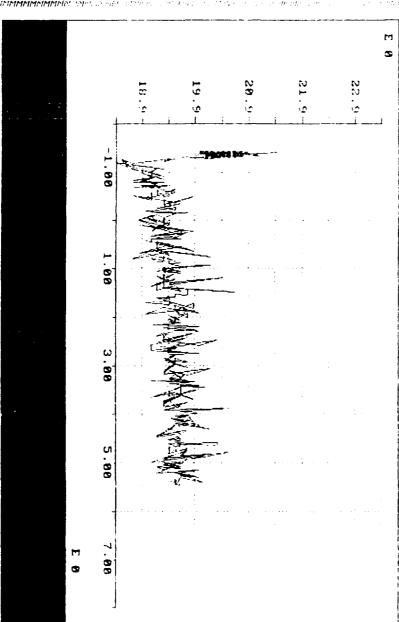
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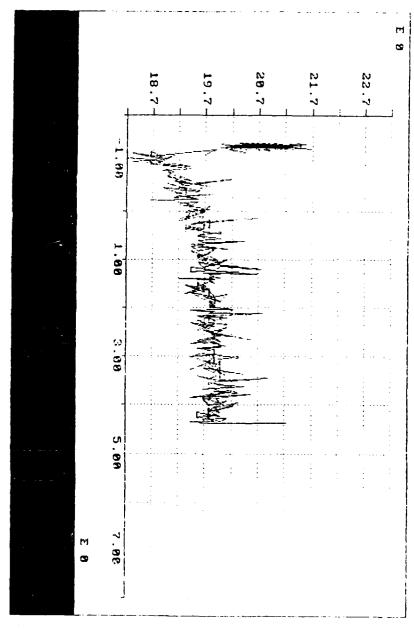
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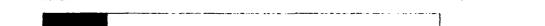
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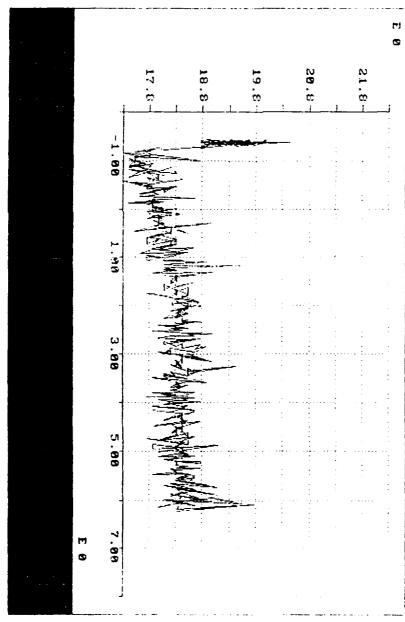


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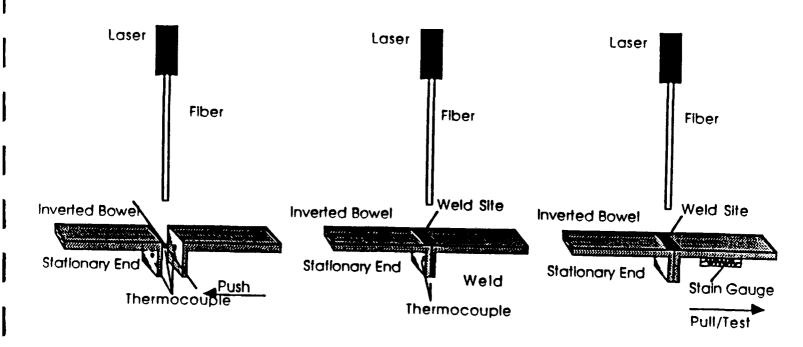
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**Appendix C- Supplemental Figures and Illustrations** 

# Figure 1- Thermocouple Placement



# Figure 2- Experimental Protocols

## LASER TISSUE WELDING OPTIMIZATION PROTOCOL

| Time (s) | Aperture Size (mm) | Power (W) | Chromophore |
|----------|--------------------|-----------|-------------|
| 2        | $0.5 \times 4.0$   | 0.05      | None        |
| 10       | $0.5 \times 4.0$   | 0.05      | None        |
| 2        | $2.0 \times 4.0$   | 0.05      | None        |
| 10       | $2.0 \times 4.0$   | 0.05      | None        |
| 2        | $0.5 \times 4.0$   | 2.0       | None        |
| 10       | $0.5 \times 4.0$   | 2.0       | None        |
| 2        | $2.0 \times 4.0$   | 2.0       | None        |
| 10       | $2.0 \times 4.0$   | 2.0       | None        |
| 2        | $0.5 \times 4.0$   | 5.0       | None        |
| 10       | $0.5 \times 4.0$   | 5.0       | None        |
| 2        | $2.0 \times 4.0$   | 5.0       | None        |
| 10       | $2.0 \times 4.0$   | 5.0       | None        |
| 2        | $0.5 \times 4.0$   | 0.05      | India Ink   |
| 10       | $0.5 \times 4.0$   | 0.05      | India Ink   |
| 2        | $2.0 \times 4.0$   | 0.05      | India Ink   |
| 10       | $2.0 \times 4.0$   | 0.05      | India Ink   |
| 2        | $0.5 \times 4.0$   | 2.0       | India Ink   |
| 10       | $0.5 \times 4.0$   | 2.0       | India Ink   |
| 2        | $2.0 \times 4.0$   | 2.0       | India Ink   |
| 10       | $2.0 \times 4.0$   | 2.0       | India Ink   |
| 2        | $0.5 \times 4.0$   | 5.0       | India Ink   |
| 10       | $0.5 \times 4.0$   | 5.0       | India Ink   |
| 2        | 2.0 x 4.0          | 5.0       | India Ink   |
| 10       | $2.0 \times 4.0$   | 5.0       | India Ink   |
| 2        | $0.5 \times 4.0$   | 0.05      | ICG         |
| 10       | $0.5 \times 4.0$   | 0.05      | ICG         |
| 2        | $2.0 \times 4.0$   | 0.05      | ICG         |
| 10       | $2.0 \times 4.0$   | 0.05      | ICG         |
| 2        | $0.5 \times 4.0$   | 2.0       | ICG         |
| 10       | $0.5 \times 4.0$   | 2.0       | ICG         |
| 2        | $2.0 \times 4.0$   | 2.0       | ICG         |
| 10       | 2.0 x 4.0          | 2.0       | ICG         |
| 2        | 0.5 x 4.0          | 5.0       | ICG         |
| 10       | 0.5 x 4.0          | 5.0       | ICG         |
| 2        | 2.0 x 4.0          | 5.0       | ICG         |
| 10       | 2.0 x 4.0          | 5.0       | ICG         |
| 2        | 0.5 x 4.0          | 0.05      | Blood       |
| 10       | 0.5 x 4.0          | 0.05      | Blood       |
| 2        | 2.0 x 4.0          | 0.05      |             |
| 10       | 2.0 x 4.0          | 0.05      | Blood       |
| 2        | 0.5 x 4.0          |           | Blood       |
| 10       |                    | 2.0       | Blood       |
| 2        | 0.5 x 4.0          | 2.0       | Blood       |
|          | 2.0 x 4.0          | 2.0       | Blood       |
| 10       | 2.0 x 4.0          | 2.0       | Blood       |
| 2        | 0.5 x 4.0          | 5.0       | Blood       |
| 10       | 0.5 x 4.0          | 5.0       | Blood       |
| 2        | 2.0 x 4.0          | 5.0       | Blood       |
| 10       | 2.0 x 4.0          | 5.0       | Blood       |
|          |                    |           |             |

### Rabbit Animal Prep Protocol

Supplies Needed: Alcohol Preps 1 ml Tuberculin Syringe 3 ml Syringe #18 Needle #22 Needle Gemini Xylazine (Rompun) Ketaset (Ketamine) 100 ml. bag of 0.9% NaCl or 5% Dextrose in Water for intravenous use Microdrip (60 drops per ml) dispenser and tubing **Animal Clippers** Manual (non-electric) Razor Artificial Tears Ointment #22 JELCO IV Catheter Placement Unit Paper Tape Beuthanasia (sodium pentobarbital or formerly known as Sleepaway)

#### Procedure:

- 1. One day prior to desired surgery, remove food container from rabbit's cage. Leave water bottle.
- 2. Prepare tray of surgical instruments for autoclaving. Place blue cloth drape under and over instruments. Add hemoclips, needle holders, atraumatic clamps, and other specialized instruments as needed. Autocjave instruments.
- 3. Collect all supplies listed above. Place in rabbit room.
- 4. Close door to rabbit room.
- 5. Determine amount of Rompun injection. A chart which indicates the appropriate amount to inject is appended to this document.
- 6. Prepare Rompun injection. Wipe the septum of the Rompun bottle with an alcohol prep. Remove tuberculin syringe from packaging. Insert syringe into bottle and carefully dispense correct amount.
- 7. With syringe in hand, gently and quietly open door to selected rabbit's cage. Pet rabbit gently to reassure. After calming rabbit, obtain control over the animal by grasping firmly at the neck. Hold down rabbit and inject Rompun into the muscle in the hind quarter.
- 8. Close rabbit cage. Wait approximately 10 minutes for rabbit to become sedated. Rabbit may slouch or turn head to side.
- 9. Remove rabbit from cage, supporting head and neck carefully.
- 10. Transfer rabbit to sink.
- 11. Using chart in appended to this document and the actual weight of the rabbit, determine the appropriate amount of Ketaset to inject.
- 12. Prepare injection of Ketaset. Remove the 3 ml. syringe from its packaging and place the #18 needle on the end of the syringe. Wipe the septum of the Ketaset bottle with an alcohol prep, and insert syringe. Remove desired amount of Ketaset and then carefully change needles from the #18 to the #22. Grasp the rabbit firmly and inject Ketaset, using the #22 needle, into the hind quarter.
- 13. Wait approximately 10 minutes for the rabbit to become completely anesthetized. The rabbit is completely anesthetized when it can be picked up and placed on its back and does not attempt to turn itself over.
- 14. While waiting, prepare intravenous anesthetic cocktail. To the 100 ml. 0.9% NaCl bag add 4.0 ml. Rompun and 8.0 ml. Ketaset. Invert bag to mix. Hang bag on IV pole. Insert microdrip and tubing into bag and then remove plastic cover from distal end of tubing. Place three or four strips of paper tape on the IV pole for later use.
- 15. Place three or four paper towels on the countertop next to the sink and two paper towels inside the sink. Place rabbit, belly up, on the countertop.

- 16. Apply Artificial Tears to the rabbit's eyes to prevent painful drying.
- 17. Using a marker, number the rabbit by writing its identification number from the experimental protocol inside its ear
- 18. Use electric clippers to shave appropriate area of rabbit, usually abdomen and groin. Push excess fur onto the paper towels. Quickly remove paper towels and fur as soon as clipping is completed.
- 19. With non-electric razor, gently remove hair from the ears, particularly along the external vein. This vein will be used for the intravenous anesthetic.
- 20. Transfer the rabbit to the surgical site.
- 21. Wipe the external ear vein region with an alcohol prep. This should improve visualization of the vein.
- 22. Remove JELCO catheter from its packaging. Insert catheter into the external vein. Use needle to enter vein and then pull back on the needle so that the catheter slides along the inside of the vein. Watch catheter slide inside the vein. Look for a backflow of blood into the top part of the catheter placement unit. Remove the needle completely when the catheter has been correctly positioned.
- 23. Attach the tubing from the IV bag to the top part of the catheter placement unit. Test catheter placement by slowly opening the anesthetic flow regulator and watching for anesthesia flow. Alternatively, open the saline regulator if one has been piggy-backed, and watch its flow. When adequate placement is verified, carefully tape the tubing and the catheter placement unit to the rabbit's ear. Tape excess tubing to the table.
- 24. Adjust the anesthetic flow rate to 1 drop every 4 seconds. Watch the rabbit's chest rise and pupil size to monitor anesthesia. If rabbit begins to come out of the anesthesia, increase rate to 1 drop every 2 seconds for about 1 minute. Return to 1 drop every 4 seconds, and wait until rabbit is sedated (1-2 minutes).
- 25. If rabbit is to be euthanized, inject 4.0 ml. of 1:1 diluted Beuthanasia into the septum of the IV line. The bottle will indicate if it has already been diluted. If it has not been diluted, mix equal parts of concentrated Beuthanasia and 0.9% NaCl, and inject this solution into the IV line. Do not inject concentrated Beuthanasia into the IV line because it is very viscous, and therefore, it is difficult to force it out of the tubing and into the rabbit.
- 26. After rabbit has died, remove IV line and place rabbit remains into a large brown plastic bag.
- 27. Carefully wash instruments. Alconox or similar detergent should be used. Never seave instruments soaking in water. Rinse instruments and place them on a towel to dry.

#### RABBIT ANESTHESIA

INITIAL INTRAMUSCULAR DOSE OF XYLAZINE GIVEN, FOLLOWED

10 MINUTES LATER BY AN INTRAMUSCULAR DOSE OF KETAMINE.

DOSAGE BASED ON WEIGHT OF RABBIT.

| WEIGHT(LBS.) |           | KETAMINE 100 MG/ML<br>ML |
|--------------|-----------|--------------------------|
| 4            | ML<br>0.5 | 0.72                     |
| 4.25         | 0.53      | 0.77                     |
| 4.5          | 0.56      | 0.81                     |
| 4.75         | 0.59      | 0.86                     |
| 5            | 0.63      | 0.9                      |
| 5.25         | 0.66      | 0.95                     |
| 5.5          | 0.69      | 0.99                     |
| 5.75         | 0.72      | 1.04                     |
| 6            | 0.75      | 1.08                     |
| 6.25         | 0.78      | 1.13                     |
| 6.5          | 0.81      | 1.17                     |
| 6.75         | 0.84      | 1.22                     |
| 7            | 0.88      | 1.26                     |

INTRAOPERATIVE ANESTHESIA CONSISTS OF 4 MG KETAMINE + 2 MG XYLAZINE IN 50 ML DILUENT, INFUSED AT 1 DROP EVERY 4-6 SECONDS FOR A 6-8 LB. RABBIT.

### Tissue Preparation for Weld Strength Optimization Study

- 1. Make a midline incision with a #15 scalpel.
- 2. Identify and isolate the small bowel.
- 3. Place an atraumatic clamp across the mesentery at the section to be resected.
- 4. Place a hemoclip at the site of the nearest vessles to occlude blood flow.
- 5. Resect a small segment of bowel measuring approximately 5 cm.
- 6. Return the remaining in situ bowel to the abdomen.
- 7. Make a longitudinal cut in the bowel segment, and open it up. Carefully rinse the bowel segment in physiological saline to remove succus entericus.
- 8. Using the specialized cutters, cut two strips measuring  $4.0 \times 20.0$  mm. Place the two strips into the clamps, making sure that the strips are aligned relative to the marks on the clamps.
- 9. Apply a barely visible amount of chromophore with a cotton-tipped applicator. The chromophore should be applied to both tissue strips.
- 10. Position the thermocouple between the two tissue strips.
- 11. Slowly bring the two tissue strips into apposition using the computer-controlled piezoelectric motor.
- 12. Verify appropriate thermocouple placement (1 mm below surface of weld).
- 13. Set laser welding parameters in laser welding control program. Weld tissue.
- 14. Switch to the tensiometry data acquisition program. Pull tissue apart and measure the load required to rupture the weld.
- 15. Repeat steps 5-14 to prepare additional tissue strips.
- 16. Import data into Lotus or Symphony to generate load versus distance plots.